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UNIVERSITY OF ALBERTA

# AN ALARM RESPONSE IN THE NORTHERN ABALONE, *HALIOTIS KAMTSCHATKANA*

BY

KATHRYN ANN COOK

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

Edmonton, Alberta
SPRING 1992

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ISBN 0-315-73177-X

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DEGREE: MASTER OF SCIENCE

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled AN ALARM RESPONSE IN THE NORTHERN ABALONE, *HALIOTIS KAMTSCHATKANA* submitted by KATHRYN ANN COOK in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE.

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## ABSTRACT

The northern abalone, *Haliotis kamtschatkana* exhibited an alarm response to the scent of damaged conspecific tissue and to mucus actively released by handled conspecifics under laboratory and field conditions. The alarm response was characterized by agitated running behaviour. This response may serve to reduce predator efficiency.

Abalones were consistently more responsive to alarm stimuli when tested in the field than they were when tested under laboratory conditions. Evidence suggests that habituation to the alarm stimulus may have occurred following repeated stimulation under laboratory conditions.

Exposure to homogenized conspecific tissue extracts revealed that the source of the alarm stimulus was associated with the mucus producing hypobranchial gland. The alarm substance is probably multifunctional and serves as more than just as a conspecific warning device. *H. kamtschatkana* has probably evolved the ability to detect a metabolite associated with the hypobranchial gland that may serve other functions in the living animal.

Spawning resulted in reduced responsiveness under laboratory conditions on two separate occasions with different animals. This decrease in responsiveness may have been the result of physical exhaustion following spawning.

Exposure to damaged tissue from taxonomically related and distant species with which *H. kamtschatkana* co-occur and do not co-occur provided evidence to suggest that *H. kamtschatkana* may also have evolved a response to heterospecific stimuli that may serve to reduce predator efficiency. These responses may be due to either ecological or taxonomic relationships or both.

## ACKNOWLEDGEMENTS

This thesis would not have been possible without the help and encouragement of many people. First and foremost, I would like to thank my thesis supervisor, Dr. Rich Palmer for his direction, advice and unrelenting patience. Gratitude is also extended to my committee members, Dr. Jeff Goldberg and Dr. John Spence for their time and helpful comments. Financial support was provided by a Graduate Research Assistantship from the University of Alberta and Graduate Student Scholarships from the Bamfield Marine Station.

Thank you to all my abalone diving buddies and boat tenders, especially Steve Lacasse, Jacquie Lee, Kathy Durante, Don Levitan, Mary Sewell, Greg Jensen, Laura Taylor, Al Benton and Nancy Sanders. Larry Green shared his knowledge of abalone care and handling. Kathy Durante, Louis Gosselin, Greg Jensen, Don Levitan, Andre Martel, Tim Rawlings, Mary Sewell and many others provided valuable comments throughout this study. Dave Hutchinson and Nancy Sanders helped with computer graphics. Jeanne Ferris helped with editing of earlier drafts and was a source of continuing support throughout the evolution of this endeavour. I am indebted to the staff at the Bamfield Marine Station for providing facilities as well as time and expertise above and beyond the call of duty.

A special thank you is extended to Tim Rawlings for his valued friendship and persistent, yet judicious prodding to get me back into the lab at night during the long, wet Bamfield winter of 1991-92.

Last, but not least, I would like to thank my parents, Herb and Kay for their emotional and financial support.

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# CHAPTER 1
# INTRODUCTION

Many aquatic organisms exhibit alarm responses to chemical substances passively released from damaged conspecifics. These include fishes (von Frisch, 1938, as cited by Smith, 1977; Pfeiffer, 1962, 1963, 1974; Smith, 1977, 1982, 1986), sea stars (Lawrence, 1991), amphibian tadpoles (Hrbacek, 1950, as cited by Snyder, 1967; Kulzer, 1954, as cited by Snyder, 1967), sea urchins (Snyder and Snyder, 1970; Parker and Shulman, 1986; Mann *et al.*, 1984), sea anemones (Howe and Sheikh, 1975), and a variety of gastropod molluscs (Kempendorff, 1940 as cited by Snyder, 1967; Snyder, 1967; Snyder and Snyder, 1971; Atema and Burd, 1975; Atema and Stenzler, 1977; Stenzler and Atema, 1977; Hadlock, 1980; Sleeper *et al.*, 1980; Alexander and Covich, (in press)). The term "alarm substance" (Schreckstoff) was first described by von Frisch (1941, as cited by Pfeiffer, 1963) as any substance that communicates the presence of danger, provided that it is produced and perceived intraspecifically.

The independent evolution of alarm responses across so many taxa suggests that they are adaptive (Atema and Stenzler, 1977). Alarm responses probably serve as antipredator devices (Snyder, 1967; Atema and Burd, 1975; Atema and Stenzler, 1977; Stenzler and Atema, 1977; Hadlock, 1980; Smith, 1982, 1986). For an alarm response to reduce the vulnerability to predators effectively, several criteria must be met (Snyder, 1967). First, the alarm substance must be released in suprathreshold levels during predation. Second, predators must be capable of feeding on more than one prey item during a feeding period. Third, the predator must take more time to eat a prey item than other prey require to hide. Finally, the alarm behaviour must be successful in avoiding predation.

Alarm responses described for gastropod species probably serve as antipredator devices. Snyder (1967) observed alarm responses to crushed conspecifics in 17 out of 28 species of freshwater snails. The form of the reaction in most species was increased motion followed by self-burial, or crawling out of the water. Some species showed both responses. Snail-predator interaction experiments in the laboratory revealed that snails that crawled out of the water or buried themselves were less likely to be eaten than unresponsive snails. Even partial burials appeared effective, as turtles had trouble grasping half buried snails (Snyder, 1967). *Nassarius* species reacted to crushed conspecifics with a self-burial response that likely serves as an effective defense against actively foraging crabs (Atema and Burd, 1975; Atema and Stenzler, 1977; Stenzler and Atema, 1977). The Florida apple snail, *Pomacea paludosa* also exhibited a self-burial response to crushed conspecifics (Snyder and Snyder, 1970). Individuals of *Littorina littorea* crawled into areas where they were less visible when crushed conspecifics were added to intertidal pools (Hadlock, 1980). In the laboratory, more time was required for predatory crabs to locate and attack snails in crevices than snails on rock surfaces (Hadlock, 1980). Although these alarm behaviours are probably adaptive, direct evidence for their ability to reduce predator efficiency is limited (Snyder, 1967; Atema and Burd, 1975; Atema and Stenzler, 1977; Stenzler and Atema, 1977; Hadlock, 1980; Smith, 1982, 1986).

Phenotypic plasticity in snails resulting from exposure to water-borne stimuli emanating from predators feeding on conspecific snails has been described by several authors (Appleton and Palmer, 1988; Crowl and Covich, 1990; Palmer, 1990). Snails held in the presence of crabs fed conspecific snails grew more predator-resistant shells in the dogwhelks, *Nucella lamellosa* (Appleton and Palmer, 1988) and *Nucella lapillus* (Palmer, 1990) compared to snails held in the presence of crabs fed frozen fish or controls. Changes in rates of feeding and growth of these snails were also observed

(Appleton and Palmer, 1988; Palmer, 1990). Life-history shifts in the freshwater snail, *Physella virgata virgata* were observed following exposure to similar stimuli. Snails exposed to water passing over crayfish actively feeding on conspecific snails showed delayed age and size at maturation and increasd longevity over snails exposed to predator-water only and to controls. These chemically induced life-history shifts may increase the likelihood of these prey coexisting with their seasonal predators (Crowl and Covich, 1990).

In species that receive little or no visual information, chemical communication is necessary to detect and avoid predators (Hadlock, 1980). Alarm responses are elicited by chemoreception of odours produced and perceived intraspecifically (von Frisch, 1941, as cited by Pfeiffer, 1963 and Sleeper *et al.*, 1980). Alarm responses should not be confused with avoidance responses which are elicited by water-borne chemicals diffusing from a distant predator, or escape responses which result from predator contact (Phillips, 1977). Numerous examples of of molluscan avoidance and escape responses to predators have been described (see reviews by Bullock, 1953; Kohn, 1961; Feder and Christensen, 1966; Feder, 1972; Underwood, 1979). Responses to predator odour or contact are not considered in this study.

Responses to alarm substances released from conspecifics are different from responses to predator odour (Atema and Stenzler, 1977). For example the marine mud snail, *Nassarius vibex,* buries itself when exposed to damaged conspecifics (Snyder, 1967), but emerges from the sand and moves away from the odour of predatory snails (Gore, 1966). Similarly, *N. obsoletus* buries itself when exposed to damaged conspecifics but crawls out of the area when presented with the odour of the predatory green crab, *Carcinus maenas,* and shows no signs of burying (Atema and Stenzler, 1977).

Flight responses to damaged tissue from heterospecific stimuli have been

documented in several marine (Snyder, 1967; Stenzler and Atema, 1977) and freshwater (Snyder, 1967) gastropod species. Responses to heterospecific stimuli are probably due to either or both taxonomic and ecological relationships (Snyder, 1967). If two species are taxonomically related, their chemical makeup may be very similar because of common ancestry. The reaction of one species to the alarm substances of another might reflect the recent divergence from a parental stock with an alarm response. On the other hand, if two unrelated species co-occur in close sympatry they may respond to heterospecific stimuli regardless of their taxonomic relationship or the similarity of their respective alarm substances. For example, if a predator feeds non-selectively, prey living in the same habitat would benefit from responding to each other's alarm substances regardless of their taxonomic relationship. However, if predators are discriminating in their choice of prey, heterospecific responses may be maladaptive as avoidance behaviour engenders numerous costs.

Abalones, archeogastropods of the family Haliotidae (genus *Haliotis* Linnaeus, 1758) are renowned for their effective and specific escape responses to asteroid predators. The alarmed abalone raises its shell and twists it sharply from side to side while moving rapidly away from the point of contact (Montgomery, 1967; James and Nolen, 1978; Parsons and MacMillan, 1979). The presence of such a dramatic escape response to predators makes this genus a likely candidate for possessing an alarm response to the scent of damaged conspecifics.

The northern or pinto abalone, *Haliotis kamtschatkana* is the only species of abalone found in the waters of Washington State, British Columbia and Alaska. Its geographic range extends from Icy Strait, at the northern tip of Sitka Island, Alaska ($58^{0}$ N) (Paul and Paul, 1981) to Baja, California ($27^{0}$ N) (McLean, 1966 as cited by Sloan and Breen, 1988). Individuals are patchily distributed on rocky substrata from the lower intertidal zone to a depth of at least 100 m. Most of the adult population is found at depths of less than 10 m (Sloan and Breen, 1988).

The presence of dramatic escape response to asteroid predators, as well as several other factors suggests that *Haliotis kamtschatkana* may possess an alarm response to the scent of damaged conspecific tissue. These factors include the presence of several specialized movement patterns, the presence of highly mobile predators and the clumped distribution of large groups of animals making chemical communication between individuals likely.

Aside from the dramatic shell-twisting response to asteroid predators, *Haliotis kamtschatkana* is also capable of several other specialized movement patterns (Sloan and Breen, 1988). Abalones have a large muscular foot which functions as a complicated hydrostatic system and allows for rapid bursts of movement (Voltzow, 1986). For example, they can right themselves when turned upside-down on their shells. This is done by curling an edge of the foot over and attaching it to the substrate to gain enough momentum to pull over on to the foot (Minchin, 1975). They can entrap pieces of drift kelp by facing into the current, lifting the anterior half of the foot, and dropping it rapidly when contact with the kelp is made (Cox, 1962). As well, they are capable of climbing up kelp plants to graze, and when they are disturbed they retreat down the plant rather than drop off (Sloan and Breen, 1988).

*Haliotis kamtschatkana* has several mobile predators including the octopus, *Octopus dofleini* (Hartwick *et al*., 1981), the cabezon, *Scorpaenichthys marmoratus*, the wolf eel, *Anarrhichthys ocellatus*, the rock crab, *Cancer productus*, and the sea star, *Pycnopodia helianthoides* (Emmet and Jamieson, 1988).

This study was conducted at the Bamfield Marine Station on Barkley Sound, Vancouver Island, B. C. and addressed the following questions:

i) Does *Haliotis kamtschatkana* exhibit an alarm response to the scent of damaged conspecifics in the absence of predators?

ii) If so, what form does the alarm response take?

iii) What is the source of the alarm stimulus?

iv) How species-specific is the alarm stimulus?

# CHAPTER 2

# MATERIALS AND METHODS

## A. Collection and study sites

For laboratory study, specimens of *Haliotis kamtschatkana* were collected, using SCUBA, from five different locations in Barkley Sound between February and November, 1990 (Fig. 1). Field tests were conducted at three sites between September 1989 and April 1991. Collection and study sites varied due to weather conditions and the availability of animals. Following the completion of each laboratory experiment, animals were returned to the site from which they had been collected.

## B. Marking and handling

Following collection, abalones were taken directly to the Bamfield Marine Station and held in water tables supplied with flow-through sea water and aeration. They were fed the kelp, *Macrocystis integrifolia*. Each animal was sexed, measured using the conventional *Haliotis* spp. shell measurements (Sloan and Breen, 1988) (see Appendix I), and tagged using individually numbered tags glued to the shell with Sea Goin' ™ Poxy Putty. All animals were allowed to acclimatize to laboratory conditions for four to eight days prior to testing.

## C. Response to damaged conspecific tissue

### 1. Laboratory tests

To test for the presence of an intraspecific alarm response in the laboratory, experiments were conducted with abalones collected from Kirby Point in February and November, 1990; Cia Rock in February, 1990; Aguilar Point in February and June, 1990; Helby Island in April and May, 1990; and Scott's Bay in October, 1990 (see Fig. 1 for sites).

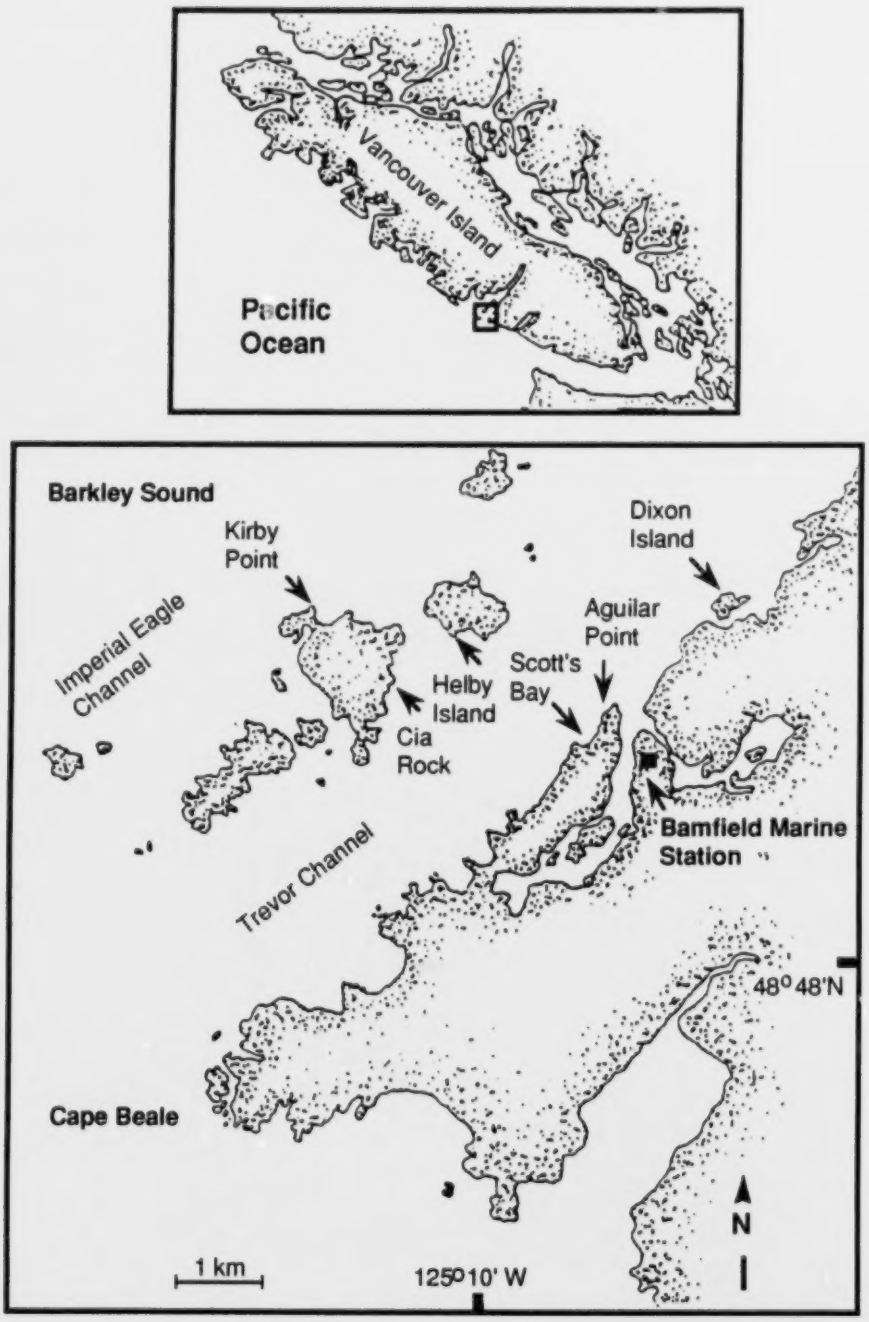



FIGURE 1. The location of collection and field sites in southeast Barkley Sound, British Columbia (modified after Martel, 1990).

The following bioassay was designed to test for the presence of the alarm response in the laboratory: Ten abalones were randomly assigned to each of eight 40 litre glass aquaria. Each tank represented one replicate. Tanks were supplied with flow-through sea water, air stones and approximately 300g wet weight of kelp. Experiments began approximately 24 hours following tank assignment. Thirty minutes prior to the beginning of an experiment the kelp was removed from the tanks to allow for clear observation. Water flow was turned off five minutes prior to the beginning of each trial, although air was supplied throughout. Preliminary dye tests revealed that the airstones caused rapid mixing throughout the tanks.

At T= zero minutes, a five minute control period began. Each individual's behaviour was recorded every 30 seconds over this five minute period. A total of six different behaviours (modified after Parsons and MacMillan, 1979) were noted. 1. *Resting*. The animal is stationary, or moving slowly with its shell slightly elevated. The cephalic and epipodial tentacles protrude only slightly from beneath the shell margin and move slowly, if at all. 2. *Tentacular sweeping*. This behaviour is characterized by full extension of the cephalic tentacles which sweep about in front of the animal, usually up and down in an alternating motion. 3. *Mushrooming*. To mushroom, the abalone elongates the central muscle column and the shell is raised clear of the substratum. 4. *Running*. This behaviour is characterized by rapid and agitated movement around the tank, sometimes resulting in the animal crawling above the water line and even out of the aquarium. The shell is elevated and may be held stationary or twist from side to side. The epipodium is often observed folded up and over the shell. The cephalic tentacles protrude from underneath the shell. Tentacular sweeping and mushrooming are often observed during this phase. 5. *Mucus secretion*. Here the abalone liberates a transparent blue mucus through the respiratory pores. 6. *Clamping*. This behaviour is characterized by tentacle withdrawal followed

by clamping down hard onto the substratum.

At T = five minutes the stimulus was applied to the test aquaria and the behaviour of each individual was recorded every 30 seconds over a five minute period. The stimulus was applied by cutting up a freshly killed abalone in the tank with a dive knife for five to ten seconds, or until damaged tissue was visible in the water. The control application consisted of waving an empty abalone shell at the water surface to simulate the mechanical stimulation caused by cutting up the test animal during the damaged tissue treatments. At the end of each trial the tank was drained by siphon, rinsed with fresh sea water and refilled. Kelp was replaced at this time.

The alarm response consisted of running behaviour, and had to persist for a minimum of 30 seconds during the five minute testing period to be considered a true response. The alarm response was never seen in any of the controls in all the experiments conducted in the laboratory.

Several measures were taken to control for possible effects resulting from repeat testing of individuals or loss of responsiveness in the laboratory over time. All abalones were stimulated with damaged conspecific tissue at the beginning and at the end of an experimental period. As well, experimental treatments were staggered over the experimental period so that at least two replicates of each treatment type were conducted on each day that an experiment was running. This design also ensured that each replicate group of animals was exposed to all the treatments being tested during an experimental period.

**2. Field tests**

To test for the presence of the alarm response under natural conditions, tests were conducted at Kirby Point in October and November, 1989; Aguilar Point in June, 1990; and Dixon Island between January-March, 1991 using the field bioassay. Abalones

were exposed to either a damaged conspecific treatment or a control.

The following bioassay was designed to test for the presence of the alarm response in the field:

Individual abalones were located by the use of SCUBA. Several criteria had to be met before an individual was tested:

a) Abalones had to be stationary with the shell slightly elevated and not clamped down on the substratum.

b) If the cephalic and epipodial tentacles protruded at all from beneath the shell they had to be moving slowly if at all.

c) Abalones had to be out in the open (i.e. not in a crack or crevice, or under an overhang).

d) Abalones had to be at least five metres away from visible predators.

e) Abalones had to be at least five metres away from a previously tested individual to lessen the chance of unintentional stimulation.

The alarm stimulus was applied by cutting up a live abalone with a dive knife several centimetres from the cephalic tentacles of the animal being tested. The observation period following the introduction of the stimulus was shorter in the field (two minutes) than in the laboratory (five minutes) due to time limitations imposed by the use of SCUBA.

The alarm response was characterized by running behaviour, and had to persist for a minimum of 30 seconds. At the end of the two minute observation period the animal was sexed and measured using the conventional *Haliotis* spp. shell measurements (Sloan and Breen, 1988) (see Appendix II). This information as well as the direction of flight, and any observed changes in behaviour such as tentacular sweeping, mushrooming, mucus secretion or clamping were recorded on an underwater slate. The distances travelled over two minutes were recorded for tests conducted at Kirby Point and Aguilar Point. The control consisted of waving a dive knife several

centimetres from the abalone being tested. Abalones were tested only once.

A fresh abalone was used as a stimulus for each trial. The damaged tissue treatment was alternated with the control. Because the alarm response was almost never seen in the controls, I feel confident that little if any unintentional stimulation occurred during these tests.

### D. Preliminary experiments to test for individual differences, habituation and the effect of the laboratory environment

Preliminary data suggested that not all abalones brought into the laboratory responded to the alarm stimulus. When stimulated once daily over several days, some individuals responded repeatedly, while others never responded, or responded inconsistently.

The following experiment was designed to determine whether there were differences between individuals brought into the laboratory, and to see whether the frequency of stimulation as well as the time an individual was held in the laboratory prior to testing had any effect on its response.

Abalones were assigned to one of six repeat stimulation schedules: a) stimulated every day, b) stimulated every second day, c) stimulated every fourth day, d) stimulated every eighth day, e) stimulated every 16th day or, f) stimulated once on the 32nd day over a 32 day testing period.

The laboratory bioassay was used with the following modification: two replicate tanks each containing five animals were used for all treatments rather than eight replicate tanks containing ten animals each. All tanks were drained, rinsed and refilled daily in order to control for the cleaning procedure.

## E. Source of the alarm substance

### 1. Laboratory tests

To determine the source of the alarm substance in *Haliotis kamtschatkana*, the following tests were conducted with abalones collected from Cia Rock in April, 1990. The laboratory bioassay was used with several modifications. Abalones were exposed to filtered homogenized extracts of five different conspecific tissue types and conspecific blood dispensed into the test aquaria by syringe. Sea water of equal volumes to homogenized tissue extracts and blood served as controls. The tissues tested were: the visceral mass, the hypobranchial or mucus gland, a whole animal, the visceral mass with the hypobranchial gland removed and the foot.

Tissue extracts were prepared by homogenizing each of these tissue types in a commercial Waring blender for two minutes at high speed with enough sea water to give a final concentration of 0.025 g wet tissue/litre sea water when added to the 40 litres of sea water in a test aquarium. This concentration was used because it represented the amount of hypobranchial gland (the smallest of the five tissue types tested) available from one individual. The concentration of homogenized tissue was later doubled from 0.025 g tissue/litre of sea water to 0.05 g of tissue/litre of sea water because it was felt that a concentration of 0.025 g tissue/litre of sea water may not have been high enough to elicit the alarm response.

Conspecific blood was drawn from the anterior aorta with a 22 gauge syringe. The final concentration of blood when added to the test aquarium was 0.09 ml blood/litre. This concentration was used because one abalone yielded approximately 3.5 ml blood which, when added to a 40 litre aquarium, yielded a concentration of 0.09 ml blood/litre sea water.

The above experiment was carried out again in May and June, 1990 with animals collected from Helby Island and Aguilar Point respectively. The bioassay was further

modified by adding a test for conspecific mucus and by doubling the concentration of the homogenized tissues. Conspecific mucus released from handled but undamaged conspecifics was dispensed by syringe into the test aquarium to give a final concentration of 0.75 ml mucus/litre of sea water. This concentration was used because one abalone yielded approximately 30 ml of mucus which, when added to a 40 litre aquarium, yielded a concentration of 0.75 ml mucus/litre sea water.

Spawning occurred within all replicate tanks midway through the trials in May and June, 1990. Trials were not run on days when spawning was observed, but all tanks were drained and rinsed thoroughly to remove all visible traces of spawn. Spawning was observed on three separate occasions in May and once in June. Each spawning event lasted two to four hours. During this time the abalones were very active and several animals crawled out of the tanks. Females produced large clouds of green eggs at irregular intervals while males produced more regular emissions of milky white spawn that quickly clouded up the tanks.

### 2. Field tests

To test whether conspecific mucus elicited the alarm response in the field, I conducted additional experiments at Aguilar Point in June, 1990 and at Dixon Island between February and April, 1991. The field bioassay was used with one modification: Abalones were exposed to either mucus released through the respiratory pores of an abalone held several centimetres from the cephalic tentacles of the animal being tested, or to a control which consisted of me just being there. Mucus secretion was easily induced by picking up an abalone and squeezing it.

## F. Species specificity of the alarm response

### 1. Laboratory tests

To assess the relative importance of ecological versus taxonomic bases of the alarm

response specificity in *Haliotis kamtschatkana*, the following tests were conducted in the laboratory. Abalones were exposed to damaged tissue from taxonomically related and distant species with which they co-occurred and did not co-occur (Table 1).

The laboratory bioassay was conducted using heterospecific stimuli with several modifications. Differences in size between species, especially the snails, were taken into account by using several individuals of the smaller species. The four snail species were crushed with a hammer, and the crushed shell and tissue were placed together in the tanks. It was not possible to cut up the snails in the tanks because they retreated inside their shells. Depending on the size of the species, one to five snails were used to give an approximate wet weight of 3.5 g snail tissue. The barnacles were also crushed with a hammer, and approximately 3.5 g tissue was added to the test tanks. Individual chitons, bivalves, and sea urchins were cut up, one per tank per trial.

**2. Field tests**

To test for flight responses to heterospecific stimuli in the field, experiments were conducted at Kirby Point in October and November 1989, and at Dixon Island between January and April, 1991. The field bioassay was used with different species as stimuli. The species tested varied between sites (Table 1).

Differences in size between the two limpet species tested at Dixon Island were taken into consideration. *Diodora aspera* is approximately two times larger than *A. mitra,* and therefore is likely to liberate twice the quantity of effluent of the smaller species. In order to control for possible effects caused by this difference in size two *A. mitra* were used to every one *D. aspera.* For the remaining species, only a single animal was used per trial.

TABLE 1. Species used to test for the presence of the alarm response in *Haliotis kamtschatkana* under laboratory and field conditions. L = laboratory experiments, F1 = field experiments conducted at Kirby Point, F2 = field experiments conducted at Dixon Island.

| Taxonomic grouping | Co-occurring with *Haliotis kamtschatkana* (subtidal species) | Not co-occurring with *Haliotis kamtschatkana* (intertidal species) |
|---|---|---|
| PHYLUM MOLLUSCA | | |
| Class Gastropoda | | |
| Subclass Prosobranchia | | |
| Order Archeogastropoda | | |
| Suborder Pleurotomariina | | |
| Family Fissurellidae | *Diodora aspera* (F2) | |
| Suborder Trochina | | |
| Family Turbinidae | *Astraea gibberosa* (L, F1) | |
| Family Trochidae | | *Tegula funebralis* (L) |
| Order Patellagastropoda | | |
| Suborder Nacellina | | |
| Family Acmaeidae | *Acmaea mitra* (F2) | |
| Order Neogastropoda | | |
| Suborder Rachiglossa | | |
| Family Muricidae | *Ceratostoma foliatum* (L) | *Nucella emarginata* (L) |
| Class Polyplachophora | | |
| Order Neoloricata | | |
| Suborder Chitonina | | |
| Family Lepidochitonidae | *Tonicella lineata* (L) | |
| Family Mopaliidae | | *Katharina tunicata* (L) |
| Class Bivalvia | | |
| Order Ostreoida | | |
| Suborder Pectinina | | |
| Family Pectinidae | *Hinnites giganteus* (L) | |
| Order Mytiloida | | |
| Family Mytilidae | | *Mytilus californianus* (L, F1, F2) |

TABLE 1. Continued

| Taxonomic grouping | Co-occurring with *Haliotis kamtschatkana* (subtidal species) | Not co-occurring with *Haliotis kamtschatkana* (intertidal species) |
|---|---|---|
| PHYLUM ARTHROPODA | | |
| SUBPHYLUM CRUSTACEA | | |
| Class Cirripedia | | |
| Order Thoracica | | |
| Suborder Balanomorpha | | |
| Family Balanidae | *Balanus nubilus* (L) | |
| PHYLUM ECHINODERMATA | | |
| Class Echinoidea | | |
| Subclass Euechinoidea | | |
| Order Echinoida | | |
| Family Strongylocentrodidae | *Strongylocentrotus franciscanus* (L, F2) | |
| Class Holothuroidea | | |
| Order Aspidochirotida | | |
| Family Stichopodidae | *Parastichopus californicus* (F2) | |

## G. Data analysis

### 1. Laboratory tests

The laboratory experiments were designed to test for differences between treatments using analysis of variance. However, departures from the assumptions of analysis of variance, including heteroscedastic variances between replicates and non-normal data, rendered this analysis invalid. These departures could not be rectified by transforming the original data. Therefore, the data were instead pooled across all replicates for each treatment type. The proportion of animals showing the alarm response was compared to the proportion of animals that did not show the alarm response. This was done by means of contingency table analyses (Statview 512+$^{tm}$ Ver. 1.2). Pairwise comparisons between treatments were made using Tukey-type multiple comparison tests for proportions on angularly transformed data (Zar, 1984; pp. 401-402).

### 2. Field tests

For field data, all animals tested were scored as showing either the alarm response or no response. Contingency table analysis (Statview 512+$^{tm}$ Ver. 1.2) was used to compare the proportion of animals responding during control versus treatment groups for each treatment type. Tukey-type Multiple comparison tests for proportions were used on angularly transformed data (Zar, 1984. pp. 401-402) to determine differences between treatments.

# CHAPTER 3

# RESULTS

## A. Form of the alarm response

### 1. Laboratory tests

The alarm response of *Haliotis kamtschatkana* was characterized by a distinctive running behaviour. The shell was lifted, the cephalic tentacles protruded from underneath the shell and the animal moved rapidly around the aquarium in an agitated manner. Occasionally an abalone would climb right out of the tank following exposure to the stimulus. Abalones move using direct ditaxic locomotory wave patterns which are restricted to the outer edges of the foot (Voltzow, 1986). Running is achieved by increasing the frequency and amplitude of these locomotory waves. Changes in the amplitude and frequency of these wave patterns were evident, but not quantified during the running phase. The outer edge of the foot was often raised several millimetres above the substratum. During the slower more relaxed crawling phase, the outer edges of the foot were barely lifted clear of the glass.

Tentacular sweeping (extension and sweeping of the cephalic tentacles in an alternating up and down motion) and mushrooming (elongation of the central muscle column which raised the shell clear of the substrate) were often observed during the running phase. As well, mucus secretion and clamping were occasionally observed. These behaviours were not considered alarm responses unless they were accompanied by running. No directional preference within the test aquaria was evident during the running phase. This was probably because mixing within the tanks was so rapid. Resting behaviour was usually restored within several minutes after the tank was rinsed and refilled with fresh sea water.

**2. Field tests**

Abalones responded noticeably more quickly and consistently in the field than in the laboratory. The alarm response was often seen within seconds of cutting into the conspecific. The alarm response observed in the field was similar to that observed in the laboratory with the exception that abalones consistently ran away from the source of the stimulus. The alarmed abalone lifted the anterior portion of the foot, twisted $90^0$-$180^0$ from the source and ran in a relatively straight line.

Although distances travelled over two minutes following exposure to the stimulus were recorded for some trials, these distances are not reported because they varied considerably among individuals (but see Appendix III). This high variability was probably due to the very heterogeneous bottom topography of the three study sites. Each animal was faced with a different array of obstacles in its flight path, including rocky pinnacles, cobble, kelp holdfasts and other invertebrates.

Alarmed abalones often stopped running and resumed the resting or clamping behaviour once in the shelter of a crack or crevice, or under the canopy of sea urchin spines. Sudden changes in direction were common. When an abalone ran into an impassable obstacle it would often lift the anterior portion of the foot and twist to change direction and then resume running. However, this was not always the case. Abalones were also observed to clamp down on the substratum upon coming to an impassable object, or while out in the open.

When abalones were picked up for sexing and measuring following a trial in the field, clouds of transparent blue mucus were often released through the respiratory pores. This behaviour was observed in almost all individuals regardless of whether they were exposed to the damaged tissue or the control application. Thus, handling alone was usually sufficient to cause the secretion of mucus. Mucus secretion was occasionally seen in the laboratory when animals were handled, but it was observed

much more frequently in the field. This mucus was different from spawn which is also released in clouds through the respiratory pores. Male spawn is milky white and female spawn is dark green.

## B. Response to damaged conspecific tissue

### 1. Laboratory tests

*Haliotis kamtschatkana* exhibited an alarm response in the laboratory. The proportion of abalones that showed an alarm response to damaged tissue differed significantly from controls for animals collected from Cia Rock, Kirby Point, Aguilar Point, Helby Island, and Scott's Bay, and the five sites combined (Table 2, Fig. 2). The alarm response was not observed in any of the controls. Heterogeneity testing of the 2 x 2 contingency tables (Zar, 1984. p. 67-68) in Table 2 comparing the Chi-square values from the five sites to a Chi-square value resulting from a contingency table formed from the pooled data confirmed that these five sites were homogeneous (heterogeneity $X^2 = 7.8$, 4df, $0.10 > P > 0.05$) and therefore justified pooling the data. However, the proportion of *H. kamtschatkana* that responded between sites was significantly different ($X^2 = 28.27$, 4df, $P = 0.0001$) when only the damaged tissue treatment was considered. Tukey-type multiple comparison tests between collection sites confirmed that abalones collected from Cia Rock, Kirby Point and Aguilar Point showed significantly higher responses to the stimulus than did those abalones collected from Helby Island and Scott's Bay (Fig. 2).

### 2. Field tests

Abalones were more responsive to the scent of damaged tissue in the field than in the laboratory ($X^2 = 30.64$, 1df, $P = 0.0001$) (data from Tables 2 and 3). Under laboratory conditions only 44% (222/500) of the animals tested showed the alarm response (Table 2), while 88% (43/49) of the animals tested in the field showed the

TABLE 2. Alarm responses of *Haliotis kamtschatkana* to damaged tissue from a whole conspecific versus a control under laboratory conditions. Abalones were collected from five different sites on separate dates. Chi-square values resulted from comparing the damaged conspecific tissue treatments with the controls by means of a 2 X 2 contingency table analysis. These data represent the results from pre-experimental trials (see Appendix IV). Data are pooled for each collection site.

| Site / Treatment | Alarm response | No response | df | $X^2$ |
|---|---|---|---|---|
| Cia Rock | | | | |
| Damaged conspecific | 25 | 15 | | |
| Control | 0 | 40 | 1 | 36.36 * |
| Kirby Point | | | | |
| Damaged conspecific | 71 | 49 | | |
| Control | 0 | 120 | 1 | 100.83 * |
| Aguilar Point | | | | |
| Damaged conspecific | 43 | 37 | | |
| Control | 0 | 100 | 1 | 58.80 * |
| Helby Island | | | | |
| Damaged conspecific | 59 | 101 | | |
| Control | 0 | 160 | 1 | 72.34 * |
| Scott's Bay | | | | |
| Damaged conspecific | 24 | 56 | | |
| Control | 0 | 80 | 1 | 28.24 * |
| All sites combined | | | | |
| Damaged conspecific | 222 | 258 | | |
| Control | 0 | 500 | 1 | 298.98* |

* $P < 0.001$

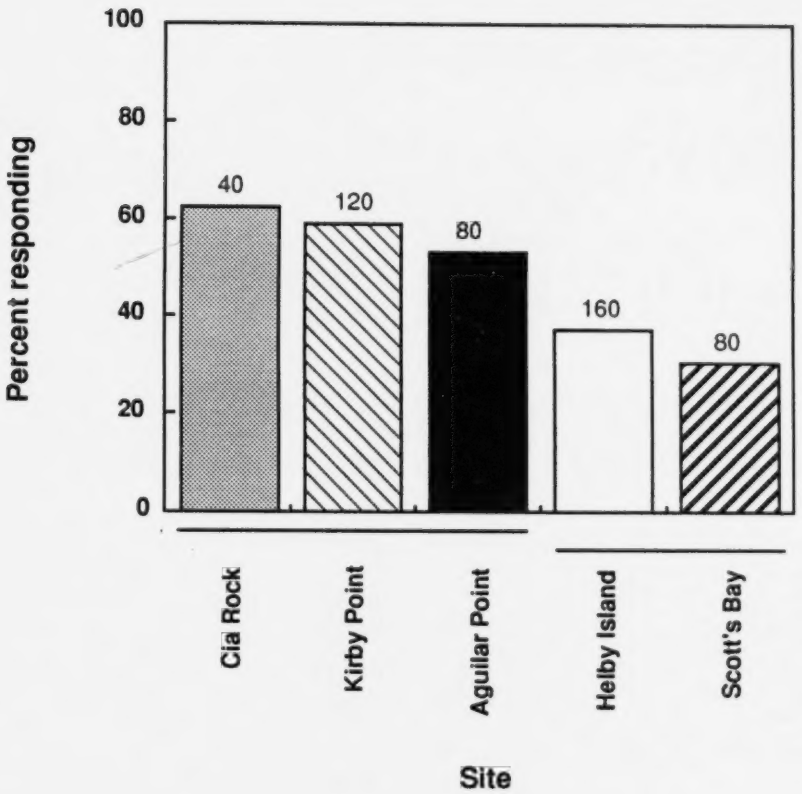



FIGURE 2. Percent of *Haliotis kamtschatkana* that showed the alarm response within five minutes following exposure to damaged conspecific tissue under laboratory conditions. Animals were collected from five different sites. Number above each bar indicates the sample size. Sites underlined were not significantly different from each other ($P > 0.05$, Tukey-type multiple comparison tests for proportions). These data represent the results from pre-experimental trials (see Appendix IV). Data are pooled for each collection site.

TABLE 3. Alarm responses of *Haliotis kamtschatkana* to damaged conspecific tissue versus a control in situ. Data were collected at Aguilar Point in June, 1990, Dixon Island between January and March, 1991 and Kirby Point in October and November, 1989. Chi-square values resulted from contingency table analysis comparing the damaged conspecific tissue treatments with the controls for each site and the three sites combined.

| Site / Treatment | Alarm response | No response | df | $X^2$ |
|---|---|---|---|---|
| Aguilar Point | | | | |
| Damaged conspecific | 20 | 2 | | |
| Control | 1 | 6 | 1 | 15.61 * |
| Dixon Island | | | | |
| Damaged conspecific | 12 | 3 | | |
| Control | 0 | 23 | 1 | 28.89 * |
| Kirby Point | | | | |
| Damaged conspecific | 11 | 1 | | |
| Control | 1 | 8 | 1 | 13.63 * |
| Aguilar Point, Dixon Island and Kirby Point pooled | | | | |
| Damaged conspecific | 43 | 6 | | |
| Control | 2 | 37 | 1 | 59.33 * |

* $P < 0.001$

alarm response (Table 3).

The proportion of *Haliotis kamtschatkana* that responded to the scent of damaged conspecific tissue in the field was significantly higher at Aguilar Point, Dixon Island, Kirby Point and the three sites combined (Table 3, Fig. 3) than the controls. Heterogeneity testing confirmed that these three samples were homogeneous (heterogeneity $X^2$= 3.20, 2df, $0.25 > P > 0.10$), and therefore justified pooling the data from the three sites. Tukey-type multiple comparison tests for proportions revealed that there were no significant differences between sites in the proportion of abalones responding to the conspecific tissue treatments or to the control (Fig. 3).

### C. Preliminary experiments to test for individual differences, habituation and the effect of the laboratory environment

These experiments showed that not all abalones brought into the laboratory responded to the alarm stimulus, and those that did respond often did not do so consistently when exposed to different repeat stimulation schedules (Figs. 4 and 5). To separate individuals based on the consistency of their responses the following arbitrary grouping of animals was used. An individual was categorized as a "responder" if it exhibited the alarm response in more than half of the trials during the first 16 days of the experiment. The rest were categorized as "non-responders." (i.e. they responded less than half the time). Animals stimulated every eight days, every 16 days, and once after 32 days were not separated in this manner due to the low number of trials.

The presence of an alarm response during the first exposure to the stimulus appeared to be a fairly reliable indication that an individual would be classified as a "responder." Twelve of the 14 individuals that were classified as "responders" based on their performance over the entire experiment showed the alarm response during first exposure to the stimulus. Only three of the sixteen animals classified as "non-

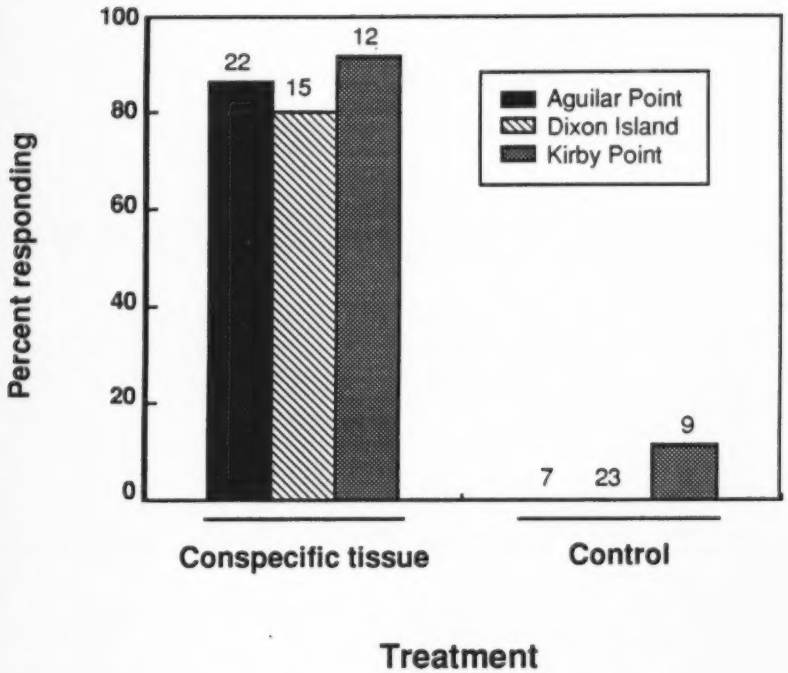
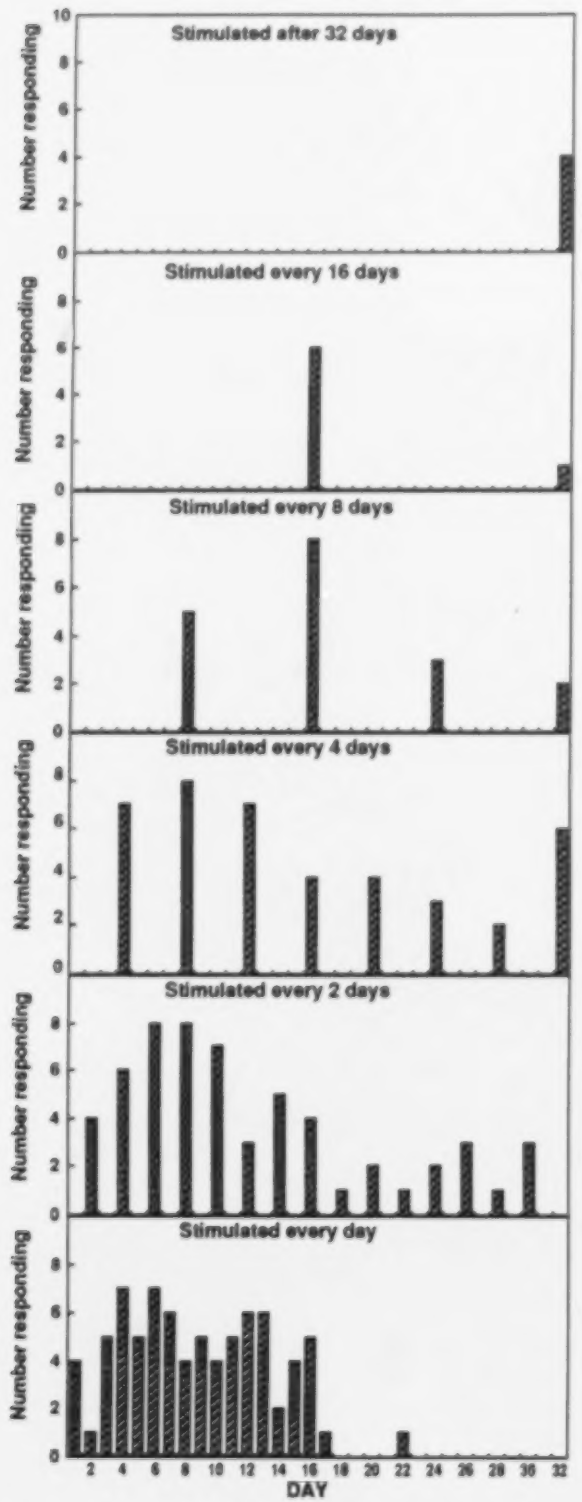



FIGURE 3. Percent of *Haliotis kamtschatkana* that showed the alarm response during a two minute period of continuous exposure to either damaged conspecific tissue or a control application in the field. Experiments were conducted at Aguilar Point, Dixon Island and Kirby Point over a two minute period. Each abalone was tested only once. The number above each bar indicates the number of abalones tested at each site. Sites underlined were not significantly different from each other ($P > 0.05$, Tukey-type multiple comparison tests for proportions).


Stimulated after 32 days
Stimulated every 16 days
Stimulated every 8 days
Stimulated every 4 days
Stimulated every 2 days
Stimulated every day
Number responding
0
2
4
6
8
10
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
DAY

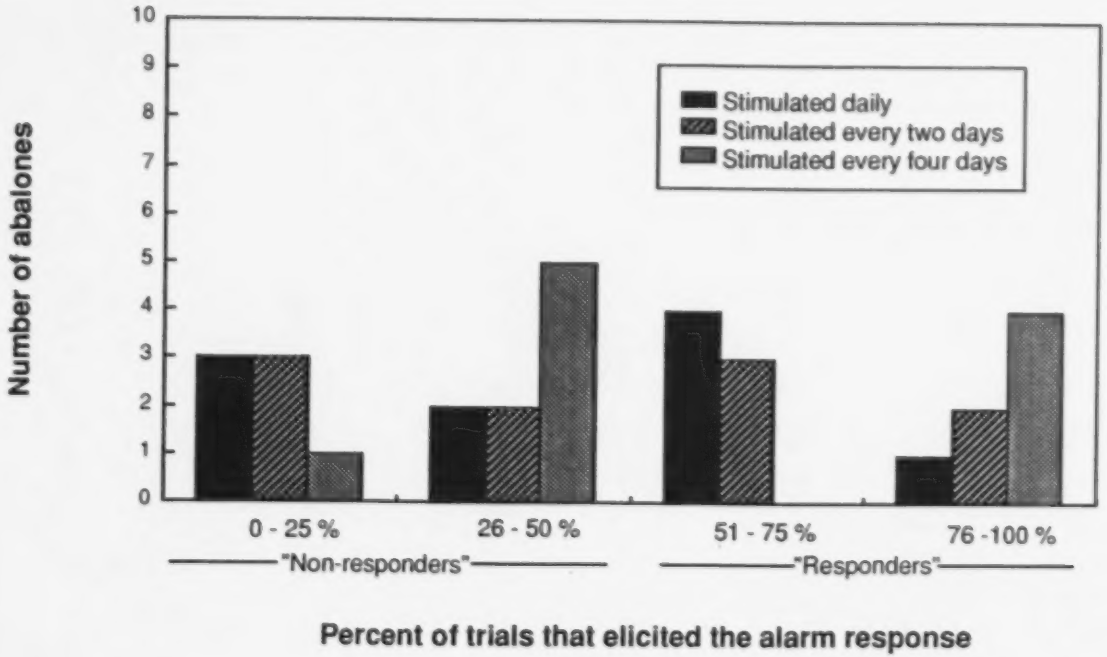



FIGURE 5. The distribution of *Haliotis kamtschatkana* that showed the alarm response 0-25 %, 26-50 %, 51-75 % or 76-100% of the time when stimulated daily, every two days or every four days with damaged conspecific tissue. Abalones that responded during 50 % or less of the trials tested were categorized as "non-responders." Abalones that responded during more than 50 % of the trials tested were categorized as "responders." N= ten abalones per stimulation schedule.

responders" responded during the first exposure to the stimulus. Therefore, two days prior to each subsequent experimental period, abalones were exposed to damaged conspecific tissue and categorized as either "responders" if they exhibited the alarm response, or "non-responders" if they did not. This procedure allowed for potential "responders" and "non-responders" to be evenly distributed among replicate tanks.

Individuals that did respond consistently did so for approximately 14-16 days of daily stimulation (Fig. 4), indicating that habituation to the stimulus did not occur until after two weeks of exposure. For this reason, laboratory experiments requiring daily stimulation were limited to a maximum of 14 days.

The time an individual was held in the laboratory prior to testing appeared to have no clear effect on its response. "Responders" or "non-responders" appeared not to differ based on sex or size when separated into either small (50-85 mm shell length) or large (86-120 mm shell length) size classes (Table 4).

Sensitization to the alarm stimulus may have occured over the course of the experiment. A clear trend of increased responsiveness over the first couple of exposures to the stimulus was evident in the every day, every two day, every four day and every eight day stimulation schedules.

To address the question of whether habituation to the stimulus occurred in the laboratory, the "responder" / "non-responder" experiments were repeated in July, 1990. Both the sample size of abalones per tank and the number of replicates used were doubled. However, the experiment was terminated after six days because virtually no abalones responded to the stimuli.

## D. Source of alarm substance

### 1. Laboratory tests

Although the responses for all the treatments tested in April, 1990 were relatively

TABLE 4. Size and sex of "responders" and "non-responders". Chi-square values resulted from contingency table analysis comparing the proportion of "responders" and "non-responders" with respsect to size class and sex. Abalones were classified as either "responders" or "non-responders" based on the consistency of their responses under laboratory conditions (see Fig. 5).

| | "Responders" | "Non-responders" | df | $X^2$ |
|---|---|---|---|---|
| Size | | | | |
| Small (50-85 mm) | 8 | 7 | | |
| Large (86-120 mm) | 10 | 4 | 1 | 1.01ns |
| Sex | | | | |
| Male | 10 | 5 | | |
| female | 8 | 6 | 1 | 0.28ns |

ns $P > 0.05$

low, the proportion of abalones that exhibited the alarm response varied significantly depending on the source of the stimulus (Table 5, Fig. 6). The highest responses were elicited by the viscera, hypobranchial gland and whole animal extracts. Virtually no abalones responded to the foot, blood or viscera-minus-hypobranchial-gland treatments, indicating that the alarm stimulus is probably not found throughout all the tissues. The alarm response was not observed during any of the control periods.

Abalones were stimulated with damaged conspecific tissue one day prior to testing with the extracts from conspecifics, and seven days later after the tests were complete to ensure that they remained responsive over the testing period. The proportion of abalones that responded at the beginning (29 %) and at the end (25 %) of the experimental period did not differ significantly ($X^2 = 0.62$, 1df, $P = 0.43$).

To increase the sample size, the above experiment was repeated in May with two modifications. The concentration of the tissue extract was doubled and conspecific mucus was added to the list of treatments. Unfortunately the responsiveness in May was also low. The proportion of abalones that responded to the viscera, hypobranchial gland, and the whole animal extracts was significantly higher than that for the viscera-minus-hypobranchial-gland and the foot treatments ($P < 0.05$, Tukey-type multiple comparisons for proportions) (Table 5, Fig. 7) which is consistent with the results from April. The mucus treatment also elicited a significant response (Table 5, Fig. 7). Again, the alarm response was not observed in any of the controls.

Spawning occurred midway through the seven day testing period of the May trials. Because the different treatments were staggered over the experimental period, it was possible to separate the data into pre-spawning and post-spawning responses. After spawning, the mucus, viscera, and hypobranchial gland still produced the highest responses of all the treatment types. All other treatments resulted in virtually no responses. However, contingency table analyses comparing the pre-and post-spawn

TABLE 5. Proportion of *Haliotis kamtschatkana* that showed the alarm response following exposure to homogenized conspecific tissue extracts, blood and mucus under laboratory conditions in April, May and June, 1990. Data for May and June are separated into pre-spawn and post-spawn results because spawning occurred during both experimental periods. Chi-square values resulted from contingency table analysis between treatments and between pre and post spawn results within each treatment.

| | Pre-spawn | | Post-spawn | | $X^2$ |
|---|---|---|---|---|---|
| | Alarm response | No response | Alarm response | No response | |
| April | | | | | |
| Mucus | __+ | __+ | — | — | — |
| Viscera | 21 | 59 | — | — | — |
| Hypo. gland | 12 | 68 | — | — | — |
| Whole animal | 10 | 70 | — | — | — |
| Vis.-Hypo. gland | 0 | 80 | — | — | — |
| Foot | 0 | 80 | — | — | — |
| Blood | 1 | 79 | — | — | — |
| | $X^2 = 54.55$*** | | | | |
| May | | | | | |
| Mucus | 16 | 44 | 5 | 15 | 0.02ns |
| Viscera | 21 | 39 | 2 | 18 | 4.58* |
| Hypo. gland | 6 | 14 | 4 | 56 | 7.47* |
| Whole animal | 5 | 15 | 0 | 60 | 16*** |
| Vis.-Hypo. gland | 1 | 39 | 1 | 39 | 0ns |
| Foot | 0 | 40 | 0 | 40 | — |
| Blood | __+ | __+ | 0 | 80 | — |
| | $X^2 = 28.85$*** | | $X^2 = 35.79$*** | | |
| June | | | | | |
| Mucus | 18 | 12 | 7 | 33 | 13.49** |
| Viscera | 6 | 24 | 1 | 39 | 5.83* |
| Hypo. gland | 8 | 22 | 4 | 36 | 3.35ns |
| Whole animal | 3 | 27 | 0 | 40 | 4.18* |
| Vis.-Hypo. gland | 0 | 30 | 0 | 40 | — |
| Foot | 0 | 40 | 0 | 40 | — |
| Blood | 0 | 30 | 0 | 40 | — |
| | $X^2 = 66.27$*** | | $X^2 = 27.69$*** | | |

*P < 0.05, **P < 0.001, ***P < 0.0001, ns P > 0.05, +No data

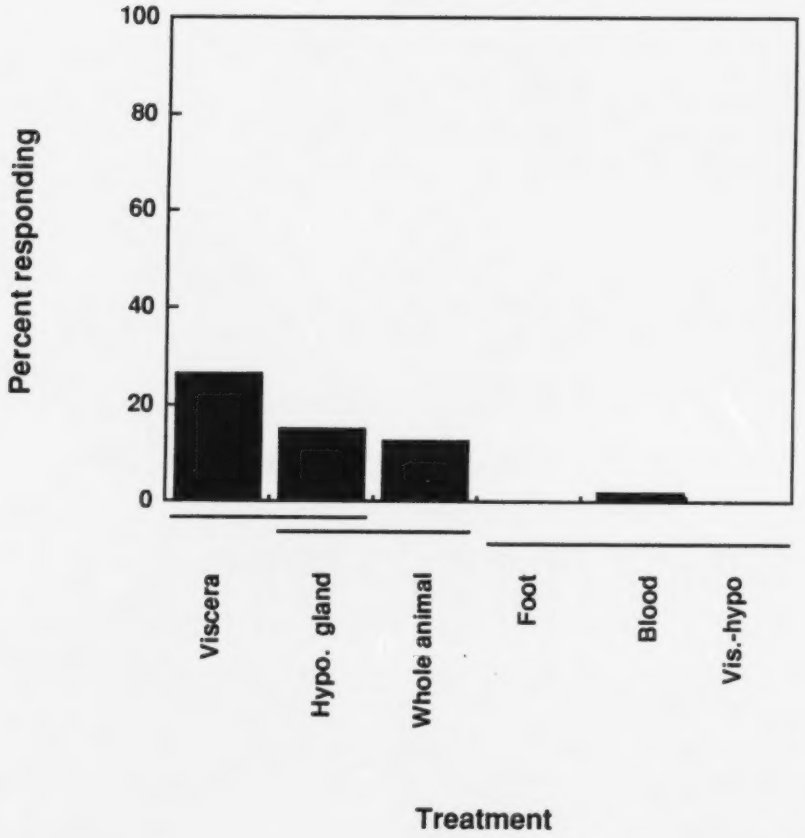



FIGURE 6. Percent of *Haliotis kamtschatkana* that showed the alarm response within five minutes following exposure to homogenized conspecific extracts (0.025 g tissue/litre sea water) and blood (0.09 ml/litre sea water) under laboratory conditions in April, 1990. N = 80 abalones per treatment. Treatments underlined were not significantly different from each other ($P > 0.05$, Tukey-type multiple comparison tests for proportions). Hypo. gland = hypobranchial gland, Vis. - hypo. = viscera with the hypobranchial gland removed.

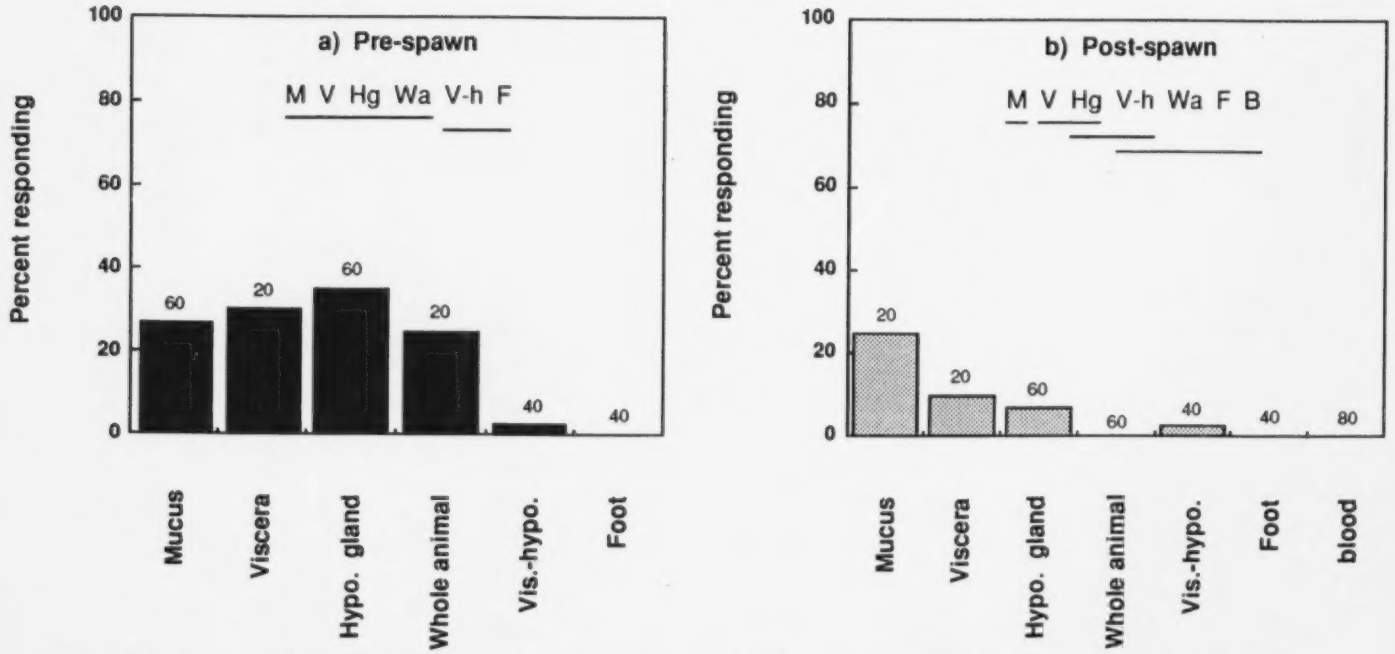



FIGURE 7. Percent of *Haliotis kamtschatkana* that showed the alarm response within five minutes following exposure to homogenized conspecific extracts (0.05 g tissue/litre sea water), blood (0.09 ml/litre sea water), and mucus released from a handled conspecific (0.75 ml/litre sea water) under laboratory conditions in May, 1990. Data are separated into a) pre-spawn and b) post-spawn results because spawning occurred midway through the seven day testing period. Number above each bar indicates the number of abalones tested. Treatment abbreviations underlined were not significantly different from each other ($P > 0.05$, Tukey-type multiple comparison tests for proportions). Hypo. gland = hypobranchial gland, Vis. - hypo. = viscera with the hypobranchial gland removed.

responses for each treatment type revealed significant differences within several treatments (Table 5). Following spawning there was a significant drop in the proportion of abalones that responded to extracts of viscera, hypobranchial gland, and the whole animal. There was no significant drop in the proportion of abalones that responded to the mucus following spawning. The proportion of abalones that responded at the beginning (45 %) and at the end (20 %) of the experimental period differed significantly ($X^2 = 11.39$, 1df, $P = 0.0007$).

The above experiment was repeated for a third time in June, 1990, using the same design as in May. The abalones again responded poorly. The results in June were very similar to those in May (Table 5, Fig. 8).

Spawning occurred again in the middle of the June experimental period. The data were therefore separated into pre-and post-spawn results (Fig. 8). As with the experiments run in May, there was a significant drop in responsiveness within several treatments following spawning. These included the mucus, viscera, and whole animal treatments (Fig. 8).

The proportion of abalones that responded to conspecific tissue at the beginning (46 %) and at the end (9 %) of the experimental period differed significantly ($X^2 = 27.76$, 1df, $P = 0.0001$).

## 2. Field tests

Mucus released through the respiratory pores of a handled abalone elicited the alarm response in the field (Fig. 9). The proportion of *Haliotis kamtschatkana* that responded to conspecific mucus versus the control was significantly higher at Aguilar Point ($X^2$=14.73, 1df, $P < 0.0001$), Dixon Island ($X^2 = 17.14$, 1df, $P < 0.0001$) and the two sites combined ($X^2$= 33.62, 1df, $P < 0.0001$) (Fig. 9). Heterogeneity

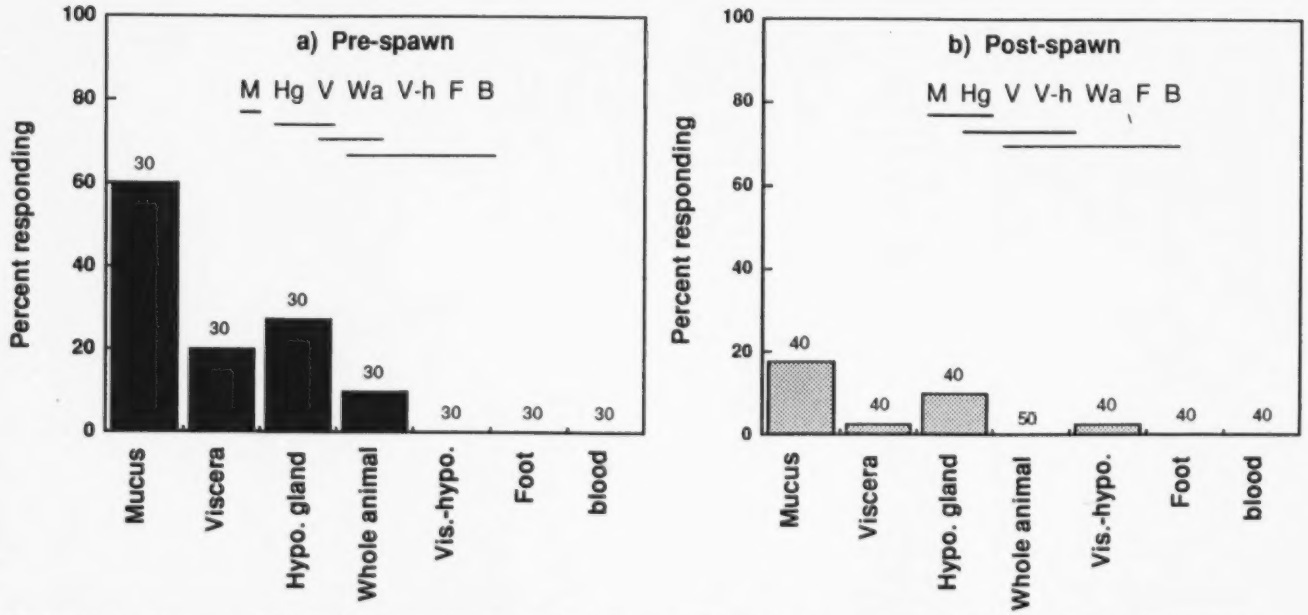



FIGURE 8. Percent of *Haliotis kamtschatkana* that showed the alarm response within five minutes following exposure to homogenized conspecific extracts (0.05 g tissue/litre sea water), blood (0.09 ml/litre sea water), and mucus released from a handled conspecific (0.75 ml/litre sea water) under laboratory conditions in June, 1990. Data are separated into a) pre-spawn and b) post-spawn results because spawning occurred midway through the seven day testing period. Number above each bar indicates the number of abalones tested. Hypo. gland = hypobranchial gland, Vis. - hypo. = viscera with the hypobranchial gland removed. Treatment abbreviations underlined were not significantly different from each other ($P < 0.05$, Tukey-type multiple comparison tests for proportions).

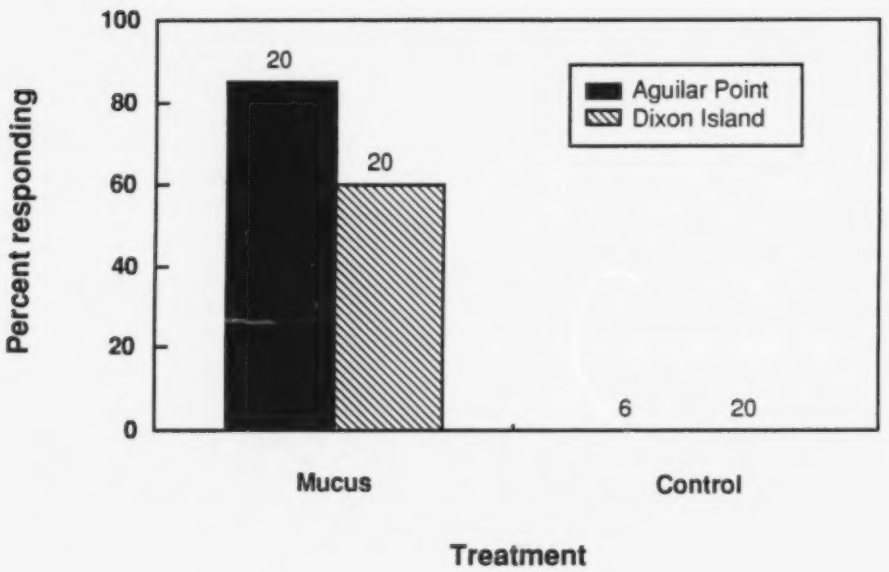



FIGURE 9. Percent of *Haliotis kamtschatkana* that showed the alarm response during two minutes of continuous exposure to actively released mucus from a conspecific or a control application conducted at Aguilar Point in June, 1990 and Dixon Island between January and March, 1991. Each abalone was tested only once. The number above each bar indicates the sample size.

testing of 2 x 2 contingency table analyses confirmed that these two sites were homogeneous (heterogeneity $X^2$=1.75, 1df, $0.25 > P > 0.10$), and therefore justified pooling the data from these two sites. Furthermore, the proportion of *H. kamtschatkana* that responded between sites was not significantly different ($X^2$ = 3.13, 1df, $0.05 < P < 0.1$) when only the mucus treatments were considered.

**E. Degradation of the stimulus**

Field tests with mucus collected from abalones held in the laboratory indicated that the alarm substance probably degrades within several hours. Field tests were conducted on two separate occasions in February and March, 1991 at Dixon Island using mucus (dispensed by syringe) which had been released upon handling from laboratory held animals collected from the Dixon Island site. Tests were carried out within three hours of active release of the mucus. Only one out of 21 animals tested over the two dives showed the alarm response.

These data were collected while conducting preliminary experiments designed to characterize the chemical makeup of the alarm substance. Attempts to conduct these tests under laboratory conditions were abandoned due to inconsistent responses. Methods for determining the chemical characterization of the alarm substance included dialysis, boiling and treating the mucus with enzymes under laboratory conditions. If consistent responses to laboratory mucus had been observed in the field, then these tests would have been conducted in the field using the laboratory altered mucus stimuli with the appropriate controls. However, because the response to mucus from laboratory held animals was not observed in animals in the field, these experiments were not done.

**F. Species specificity of the alarm response**

*Haliotis kamtschatkana* responded to the effluents from all the species tested in the laboratory (Fig. 10) and in the field (Fig. 11). Although the proportion of abalones that responded to the heterospecific stimuli in the laboratory was relatively low, some stimuli elicited a stronger response than others ($X^2 = 30.48$, 9df, $P = 0.004$, contingency table analysis). The highest percent responses were elicited by stimuli from *Astraea gibberosa, Hinnites giganteus, Tegula funebralis* and *Tonicella lineata* ($P < 0.05$, Tukey-type multiple comparison tests for proportions). Responses to these stimuli were significantly higher than to all other species tested (except *T. lineata*). The alarm response was never observed during any of the control periods (Fig. 10). The proportion of abalones that responded to damaged conspecific tissue at the beginning (70 %) and at the end (55 %) of the experimental period in the laboratory also not differ significantly ($X^2 = 1.92$, 1df, $P = 0.16$).

In the field, the proportion of *Haliotis kamtschatkana* that responded to the damaged conspecific and heterospecific stimuli varied significantly at Kirby Point ($X^2 = 7.72$, 2df, $P = 0.02$) and at Dixon Island ($X^2 = 32.86$, 5df, $P = 0.0001$) (Fig. 11). The highest responses were elicited by the closely related and sympatric keyhole limpet *Diodora aspera* at Dixon Island. This response was not significantly different from the response to *H. kamtschatkana* (Fig. 11b). Similarly, the proportion of abalones that responded to conspecific tissue and to the tissue from *Astraea gibberosa* did not differ significantly at Kirby Point (Fig. 11a).

To examine a possible taxonomic or ecological basis of responses to heterospecific stimuli, the laboratory and field data were pooled and separated according to taxonomic similarity (Table 6a) and ecological similarity (Table 6b). The species tested were separated into arbitrary groupings of high, mid and low percent responding, based on the proportion of abalones that responded for each species tested in the laboratory (Fig. 10) and in the field (Fig. 11).

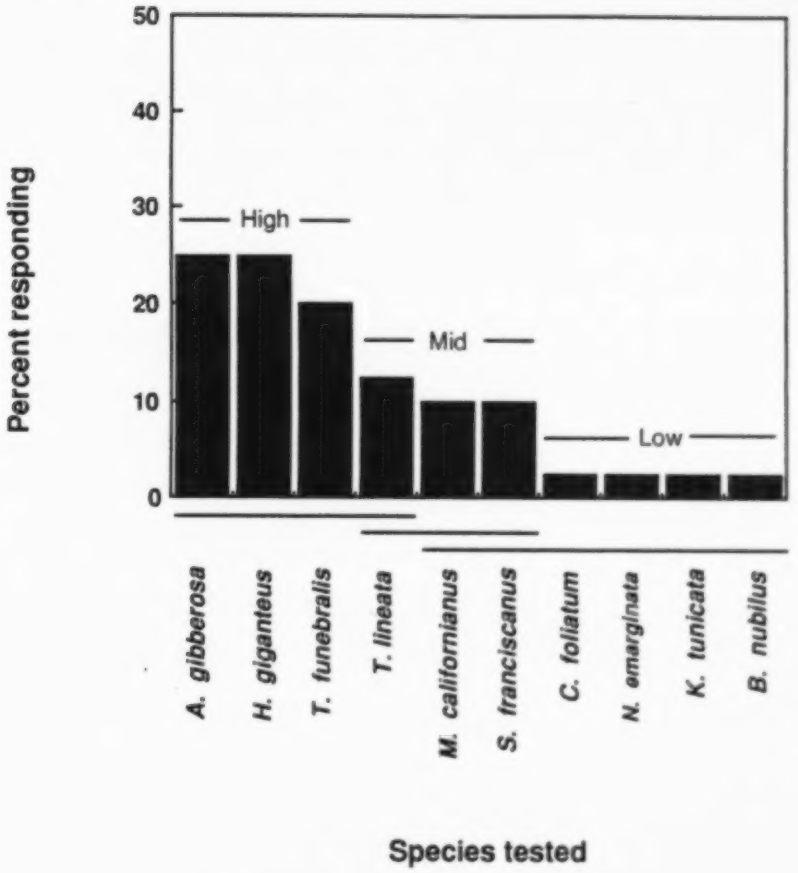



FIGURE 10. Percent of *Haliotis kamtschatkana* that showed the alarm response within five minutes following exposure to damaged tissue from each of ten different species in the laboratory. Each abalone was exposed to a different heterospecific tissue type daily over a 10 day testing period. N = 40 abalones per treatment. Treatments underlined were not significantly different from each other ($P > 0.05$, Tukey-type multiple comparison tests for proportions). Data are separated into arbitrary groupings of high, mid and low responses based on the proportion of abalones that responded to each species tested (see Tables 6a and 6b).

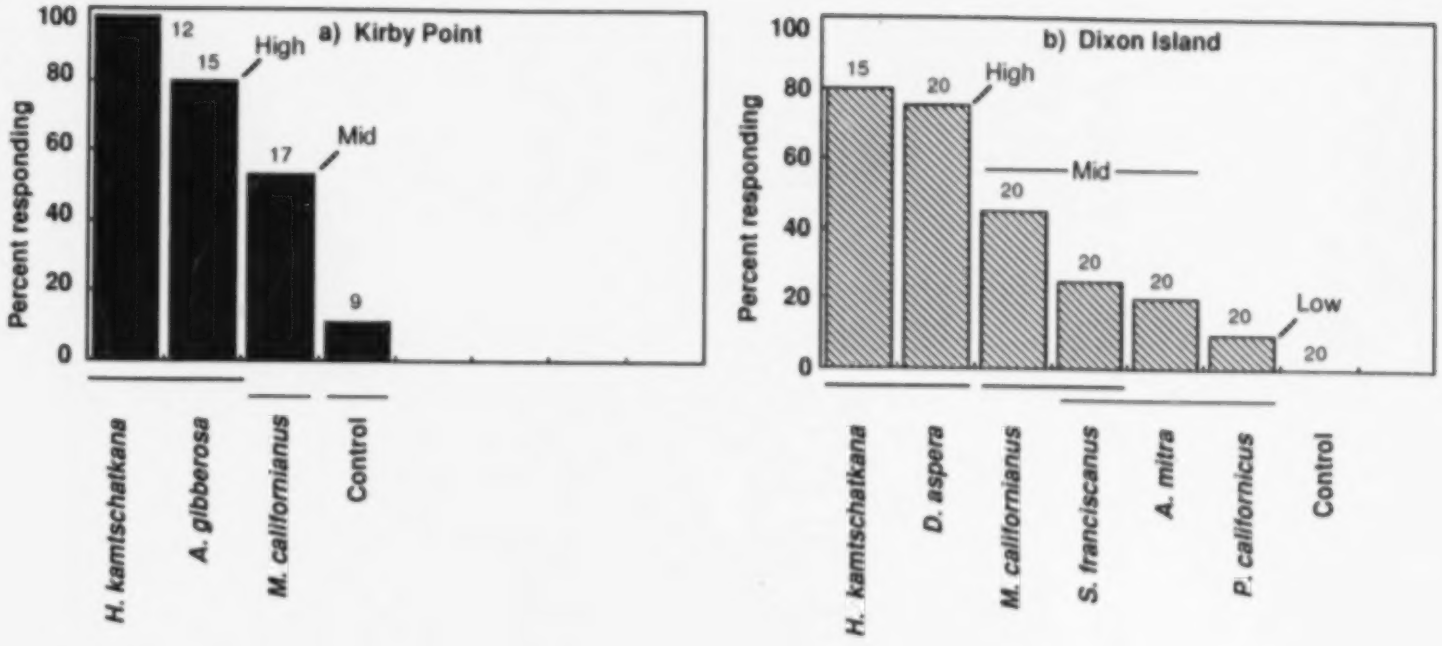



FIGURE 11. Percent of *Haliotis kamtschatkana* that showed the alarm response during two minutes of continuous exposure to 1) damaged conspecific tissue, 2) one of several different heterospecific tissues, or 3) a control application. Data were collected at a) Kirby Point and b) Dixon Island. Number above each bar indicates the sample size. Abalones were tested only once. Treatments underlined were not significantly different from each other ($P > 0.05$, Tukey-type multiple comparison tests for proportions). Data are separated into arbitrary groupings of high, mid and low responses based on the proportion of abalones that responded to each species tested (see Tables 6a and 6b).

TABLE 6a. Number of species that elicited high, mid or low percent responding in *Haliotis kamtschatkana* when separated according to taxonomic similarity to *H. kamtschatkana*. Data are pooled for sympatric and allopatric species tested in the laboratory and in the field. Arbitrary groupings of high, mid and low responses are based on the proportion of abalones that responded for each species tested (see Figs. 10 and 11).

| | Percent of abalones responding | | |
|---|---|---|---|
| Taxonomic relatedness to *H. kamtschatkana* | High | Mid | Low |
| Same Order | 3 | — | — |
| Same Class | — | 1 | 2 |
| Same Phylum | 1 | 2 | 1 |
| Other Phylum | — | 1 | 2 |

TABLE 6b. Number of species that elicited high, mid or low percent responding in *Haliotis kamtschatkana* when separated according to ecological relatedness to *H. kamtschatkana*. Data are pooled across all taxa for laboratory and field tests. Arbitrary groupings of high, mid and low responses are based on the proportion of abalones that responded for each species tested (see Figs. 10 and 11).

| | Percent of abalones responding | | |
|---|---|---|---|
| Ecological relatedness to *H. kamtschatkana* | High | Mid | Low |
| Sympatric Species | 3 | 3 | 2 |
| Allopatric Species | 1 | 1 | 3 |

With one exception, those species eliciting a high percent response were all taxonomically similar species in the same order (Table 6a). Species in different orders or classes within the Phylum Mollusca or other phyla elicited only low to mid responses.

When species were separated based on sympatric and allopatric relationships (Table 6b) sympatric species tended to elicit a higher percent responding than allopatric species, but this trend was not significant statistically ($X^2$=1.593. 2df, $P$ = 0.45).

# CHAPTER 4
# DISCUSSION

## A. Response to damaged conspecific tissue

*Haliotis kamtschatkana* exhibited an alarm response both to the scent of damaged tissue and to mucus actively released from disturbed abalones under laboratory and field conditions. The alarm response consisted of agitated running behaviour that was often accompanied by tentacular extension and sweeping as well as mushrooming. This alarm behaviour is almost identical to the escape response elicited by contact with predatory sea stars in *H. kamtschatkana* (James and Nolen, 1978), *H. assimilis*, *H. rufescens* (Montgomery, 1967) and *H. laevigata* (Parsons and MacMillan, 1979). The escape response differs from the alarm response only in that, upon initial contact with a sea star, the abalone violently twists the shell from side to side presumably to shake itself free from the predator. It is not surprising that shell twisting was not observed during the alarm response, because in the absence of a predator this behaviour would not only be energetically very costly, but would probably serve no adaptive purpose.

The alarm behaviour would seem to be an adaptive response to reduce the efficiency of a mobile predator for two reasons. First, response to the stimulus under field conditions was usually quick. Assuming that a predator can consume more than one prey item during a feeding period, a rapid response would be beneficial. Second, alarmed abalones tended to move away from the location of the stimulus in the field and often stopped running once in the shelter of a crack or crevice.

The alarm response as described in this study should result in lower rates of detection and successful attack by predators. Similarly, alarm responses described for other gastropod species also appear to be adaptive and probably result in decreased predator efficiency. However, direct evidence to show that alarm responses in

*Haliotis kamtschatkana* and in other gastropods (Snyder, 1967; Atema and Burd, 1975; Atema and Stenzler, 1977; Stenzler and Atema, 1977; Hadlock, 1980) do in fact reduce predator efficiency is limited.

## B. Preliminary experiments to test for individual differences, habituation, and the effect of the laboratory environment

Attempts to elicit the alarm response in *Haliotis kamtschatkana* under laboratory conditions gave inconsistent results. Not all abalones responded to the alarm stimuli, and those that did respond did not do so consistently when stimulated repeatedly (Fig. 5). Some evidence suggests that habituation to the alarm stimulus occurred after two weeks of daily stimulation in the laboratory (Fig. 4). Further testing was conducted, but an accurate determination of whether habituation was actually occurring under laboratory conditions could not be made due to inconsistent results.

The presence of site differences in laboratory experiments with damaged conspecifics was probably due to inconsistent responses under laboratory conditions because site differences were not evident in field tests. Inconsistent laboratory results are not uncommon when testing for alarm responses in other species. When first describing the alarm response in the European minnow, *Phoxinus laevis*, von Frisch (1941, as cited by Pfeiffer, 1963), found it necessary to perform 438 tests to establish the nature of the reaction and the source of the stimulus. After ruling out temperature, seasonal differences, collection sites, and sex of the animal as possible factors that might contribute to the variability of responses, he finally concluded that the variability of the results was dependent on the readiness of the fish to react. Fish from the same catch, exposed to the same treatment varied greatly in their responses to the same test substance. The number of times an animal was exposed to the stimulus, and the length of time an animal was allowed to acclimate to the laboratory, were cited as possible

factors influencing responsiveness. As with the present study, von Frisch found a decrease in responsiveness with repeated testing. Acclimation time also appeared to affect responsiveness. Fish acclimated for two weeks were more likely to respond to the stimulus than fish that were only acclimated for several days. Kulzer (1954, as cited by Pfeiffer, 1963), however found that acclimation time had no effect on responsiveness in tadpoles.

Snyder and Snyder (1970) also reported inconsistent results from laboratory studies with the sea urchin *Diadema antillarum*. As with the present study, an accurate determination of the threshold concentration necessary to elicit the response could not be made due to inconsistent responses.

### C. Source of the alarm substance

The alarm substance in *Haliotis kamtschatkana* did not appear to be present in all the tissues. The homogenized tissue experiments indicated that those tissues containing the hypobranchial gland elicited the most responses (Table 5, Fig. 6-8) The extracts of whole animal, whole viscera, and hypobranchial gland elicited strong responses. Extracts of viscera-minus-the-hypobranchial-gland, foot, and blood drawn directly from the pericardial cavity of a live abalone elicited very few responses.

Unlike *Haliotis kamtschatkana*, the alarm stimulus in the marine mud snail *Nassarius obsoletus* appears to be present throughout the viscera. *N. obsoletus* shows a self-burial response when exposed to homogenized extracts of conspecific foot, visceral mass, and blood (Atema and Stenzler, 1977).

The function of the hypobranchial gland in most prosobranchs is believed to be primarily for waste disposal (Hughes, 1986). Detrital particles brought in with the inhalent current adhere to mucus secretions from the hypobranchial gland. These psuedofaeces are transported by ciliary tracts across the mantle cavity to the exhalent

region where they are expelled. Other possible functions of the hypobranchial gland include the production of toxins used to overcome prey in dogwhelks (Dubois, 1909, as cited by Fretter and Graham, 1962), the production of a sexual attractant in *Nucella* (Fischer, 1925, as cited by Fretter and Graham, 1962), and the production of substances in several predatory gastropods that elicit escape responses in their prey. Laurenson (1970, as cited by Mackie and Grant, 1974) isolated urochanylcholine from the hypobranchial gland of the predatory gastropod, *Haustrum haustorum*, and identified it as the substance that causes the escape response in its prey, *Melagraphia aethiops*. Urochanylcholine has been isolated from *Murex* species, *Tritonalia erinacea*, *Urosalpinx cinerea* and *Nucella lapillus*, senecioylcholine from *Nucella floridana* and acrylycholine from *Buccinum undatum* (Whittaker, 1960 as cited by Mackie and Grant, 1974). The most widely cited function of the hypobranchial gland, however is its role in waste disposal (Fretter and Graham, 1962; Hughes, 1986).

**D. Mode of release of the alarm substance**

The alarm substance in *Haliotis kamtschatkana* appeared to be elicited by effluents passively released from injured conspecifics as well as from mucus actively released from uninjured, but handled animals (Fig. 9). Passive and active release of the alarm stimulus differ in that passive release requires tissue damage, whereas active release does not involve tissue damage. The homogenized tissue experiments indicated that the source of the passively released alarm stimulus was probably the hypobranchial gland. The hypobranchial gland may be the site of synthesis of the alarm causing mucus that is released actively through the respiratory pores of an abalone following rough handling. The major component of gastropod mucus is water followed by ash, protein and carbohydrate (Hunt, 1970; Grenon and Walker, 1980; Davies *et al*, 1990). Water comprises approximately 99 % of hypobranchial mucus in the snail *Buccinum undatum* (Hunt, 1970) and 90-92% of pedal mucus in several species of limpets

(Grenon and Walker, 1980; Davies *et al*, 1990).

Very little evidence exists for the active release of alarm substances in other species. Montgomery (1967) reported limited evidence of an actively released alarm substance in two other abalones *Haliotis assimilis* and *H. rufescens*. Following stimulation with the predatory sea stars, *Pycnopodia helianthoides* and *Pisaster ochraceus*, both species liberated mucus through the respiratory pores. When conspecific mucus was introduced into a tank with two *H. assimilis*, flight responses which consisted of movement several times normal speed were observed. The same test using *H. rufescens* resulted in slight epipodial covering of the shell and "slow flight responses". When mucus from *H. assimilis* was added to a tank containing two *H. rufescens* and vice versa the only response observed was increased tentacle activity. This work was preliminary however, and no further testing was conducted.

Active release of an alarm substance has also been documented for the marine opisthobranch, *Navanax inermis* (Sleeper *et al*., 1980). When molested, but not injured, this animal secretes a bright yellow substance into its slime trail. Upon encountering this secretion, a trail-following conspecific exhibits an avoidance response consisting of contraction, turning at an angle of greater than $90^{o}$ and retreating.

Attempts to produce the active release of the alarm substance in the mud snail, *Nassarius obsoletus* were unsuccessful (Atema and Stenzler, 1977). A mud snail was hit between two large rocks causing a visible crack in the shell. When this snail was used as a stimulus source for other conspecifics burial behaviour was not significantly different from controls. Even after removing the entire shell except for a small attachment to the columellar muscle, the snail did not elicit an alarm response when presented to conspecifics. Only when the last piece of shell was cut from the muscle was the alarm response induced, presumably by the passive release of the alarm stimulus as a result of tissue damage (Atema and Stenzler, 1977).

Snyder (1967) reported an alarm response in *Helisoma duryi* to water in which conspecifics had been repeatedly dropped. Although active release of the alarm substance was not ruled out completely, superficial injury in handling snails was cited as a more likely explanation for the release of the alarm substance.

Passive release appears to be the most common mode of release for the alarm substances in *Nassarius* species (Snyder, 1967; Atema and Burd, 1975; Atema and Stenzler, 1977; Stenzler and Atema, 1977), *Littorina littorea* (Hadlock, 1980) and a variety of freshwater gastropods (Snyder, 1967). Snyder (1967) dismisses active release of alarm substances as a general phenomenon in gastropods for two reasons. First, if a snail receives a chemical stimulus that elicits the active release of the alarm substance, this release could result in a chain reaction of alarm stimulus release by neighbouring conspecifics. This could then alarm snails at a great distance from the original source of danger. Second, a snail may lessen its own chances of survival if it releases alarm substances actively. By warning nearby conspecifics and causing them to conceal themselves a snail may in turn render itself more vulnerable to predation.

Unnecessary release of the alarm substance which might result in a chain reaction of alarm signalling as described by Snyder (1967) is probably not common in *Haliotis kamtschatkana*. Active release of the alarm substance was rarely observed in unhandled abalones. Stimulation with actively released mucus elicited the alarm response of running in conspecifics, but handling was necessary to elicit the release of mucus. Perhaps handling mimics capture or physical contact by a predator, and thus poses a real threat to the abalone. Without physical contact the alarm substance is not released, and therefore nearby conspecifics would not be falsely warned of danger.

Although Snyder (1967) argues that warning nearby conspecifics might render a snail more vulnerable to predation, this assumes that the alarm substance serves only as a warning device. If so, then it would be classified as a true pheromone as defined by

Burghardt (1970). However, it is more likely that *Haliotis kamtschatkana* has evolved the ability to detect a metabolite associated with hypobranchial mucus that probably serves another function. The idea of evolving the ability to detect and respond to a metabolite which serves another purpose in the living snail is not new. Such an alarm system has been proposed for *Nassarius* species (Atema and Stenzler, 1977), and for freshwater snails (Snyder, 1967).

Some gastropods have developed toxic mucus secretions that serve as defensive agents against potential predators (Branch, 1981). Mucus induced by handling in *Haliotis kamtschatkana* might serve as a predator irritant or toxin, and not strictly as an early warning device for conspecifics. If the mucus does serve as a predator irritant then perhaps it is also perceived simply as an irritant to conspecifics which results in the seemingly adaptive antipredator response. However, given the dramatic running response induced by the release of this mucus, this seems unlikely.

Actively released mucus may also serve to protect an abalone from predation by making the shell slippery and difficult to grasp. Following repeated stimulation with *Pycnopodia helianthoides*, *Haliotis assimilis* emitted mucus which "fouled the aboral surface of the asteroid to the point where it was felt that continuation of the experiment was useless" (Montgomery, 1967). Whether or not the actively released mucus does serve as a predator irritant is not known.

### E. Possible evolution of the alarm response

For an alarm response to evolve, a species must have the ability to detect and respond to an alarm substance in such a way that the probability of a conspecific becoming a prey item is reduced (Snyder, 1967). The results of this study show that *Haliotis kamtschatkana* has developed the ability to detect an alarm substance. The response to the alarm stimulus may reduce predator efficiency. The alarm substance is

probably not unifunctional.

If the alarm substance serves another function in the living snail, then natural selection will preserve its existence based on this other function (Snyder, 1967; Atema and Stenzler, 1977). However, if the alarm substance serves strictly as a warning device for conspecifics then the development and maintenance of the alarm substance may be an example of altruism maintained by kin selection (Smith, 1977). In other words, the sender of the alarm substance would not benefit directly from the metabolic costs incurred from the development and maintenance of the alarm substance (Atema and Stenzler, 1977).

The alarm system in ostariophysian fish may be an example of an alarm system maintained by kin selection (Smith, 1977). These fish have large epidermal cells which contain a specific alarm substance that is released when they are physically damaged (for reviews see Pfeiffer, 1962, 1963, 1974; Smith, 1982). Release of the alarm substance results in increased schooling and avoidance of the area in which the stimulus was first perceived (Pfeiffer, 1962, 1963, 1974). This behaviour is similar to that observed following contact with predators, and therefore is probably adaptive (Smith, 1982). No other function of these alarm substance cells is known (Smith, 1982). If this alarm substance serves strictly as a warning device for conspecifics then it could be considered a true pheromone (Burghardt, 1970). Although kin selection remains a possibility, the alarm substance cells in ostariophysian fish may nonetheless serve some other unknown function, and thus their evolution and maintenance may have resulted from direct selection on the sender (Smith, 1977).

### F. Pre-spawning versus post-spawning behaviour

Responding to the alarm substance did not always appear to be the highest behavioural priority in *Haliotis kamtschatkana*. Spawning resulted in reduced responsiveness under laboratory conditions on two separate occasions with different

animals. This decrease in responsiveness may have been the result of physical exhaustion following spawning. Atema and Stenzler (1977) also found that under certain conditions, the mud snail *Nassarius obsoletus* would not respond to the alarm substance. Snails would not respond when under starvation stress, or while copulating. Snyder (1967) also reported a lack of responsiveness when snails were copulating. For an alarm response to be valuable to a species, the advantage gained by reacting must outweigh the disadvantage of expending the energy necessary to react and of taking the time out from activities such as feeding and copulating (Snyder, 1967).

## G. Responses to heterospecific stimuli

The responsiveness of a species to heterospecific stimuli may reflect taxonomic or ecological relationships (Snyder, 1967, Snyder and Snyder, 1970). If two species are taxonomically related, their chemical makeup may be very similar. However, if two distantly related species co-occur in close sympatry they may respond to one another's alarm substances regardless of their taxonomic relationship. For example, if two species co-occur and share the same predators they will certainly benefit from responding to one another's alarm substances regardless of taxonomic relationships (Snyder, 1967).

Evidence exists for both taxonomic and ecological correlates of heterospecific responsiveness for fish (Pfeiffer, 1962, 1963), sea urchins (Snyder and Snyder, 1970) and snails (Snyder, 1967; Stenzler and Atema, 1977). Taxonomic correlates in fishes may exist, but ecological correlates are probably less important (Pfeiffer, 1962, 1963). Snyder and Snyder (1970) concluded that taxonomic and ecological relationships were not a reliable guide to determining stimulus potency in the sea urchin, *Diadema antillarum*. This urchin was exposed to stimuli from four distantly related urchins (different superorder or different subclass) with which it co-occurred. Three out of the

four species tested were closely related to each other, yet their potency for producing alarm in *D. antillarum* differed significantly (Snyder and Snyder, 1970). Because these urchins all lived within close proximity of each other, it was not clear that the strength of response of *D. antillarum* could be related to the extent of sympatry with these species (Snyder and Snyder, 1970).

Stenzler and Atema (1977) showed that a clear but less pronounced response to crushed congeners was elicited in *Nassarius* species. Taxonomically unrelated gastropod species caused no responses in any of the *Nassarius* except in one case. *N. trivittatus* showed a strong response to stimuli from *Urosalpinx cinerea*, which indicated a possible ecological relationship (Stenzler and Atema, 1977). *U. cinerea* co-occurs with *N. trivittatus* but not with the other *Nassarius* species. *U. cinerea* alarm substance probably differs from *Nassarius* substance based on the taxonomic distance between these two species (Stenzler and Atema, 1977). If these two species share the same predators, then detecting and responding to stimuli from *U. cinerea* would be adaptive in *N. trivittatus*.

This study provides limited evidence for both taxonomic and ecological correlates of heterospecific responsiveness in *Haliotis kamtschatkana* (Tables 6a and 6b). Species within the same order as *H. kamtschatkana* (*Astraea gibberosa*, *Tegula funebralis* and *Diodora aspera*) all elicited high percent responses. Species in different orders, classes and other phyla elicited mostly mid and low percent responses. This is not surprising as closely related species may be chemically very similar.

Evidence also exists for ecological correlates for heterospecific responsiveness. More abalones responded to tissue from sympatric species than to tissue from allopatric species (Table 6b). This might be expected as co-occurring species will certainly benefit from responding to one another's alarm substances regardless of their chemical makeup providing that they share the same predators.

However, the low percent responding to damaged tissue of the co-occurring and abundant sea urchin *Strongylocentrotus droebachiensis* and the sea cucumber *Parastichopus californicus* tested under field conditions would appear to indicate that there are no ecological relationships between these species and *H. kamtschatkana*.

Taxonomic and ecological relationships are not always good indices of a species likelihood of eliciting a response. The presence of a response to the mussel *Mytilus californianus* in the field is puzzling. It is not closely related to *Haliotis kamtschatkana*, nor does it co-occur with *H. kamtschatkana*. Interestingly, the proportion of *H. kamtschatkana* responding to damaged *M. californianus* tissue was consistent between field sites. Approximately 50 percent of the abalones from both sites exhibited responses when exposed to damaged tissue from this mussel. The chemical makeup of these two molluscan species may be similar even though they are not closely related.

The question of an ecological versus a taxonomic basis for responses to heterospecific stimuli in *Haliotis kamtschatkana* thus remains open. It is difficult to draw any conclusions on the taxonomic basis of responses to heterospecific stimuli based on the evidence presented in this study. None of the heterospecific stimuli tested in this study are very closely related to *H. kamtschatkana*. In order to determine whether responses to heterospecific stimuli in *H. kamtschatkana* have a taxonomic basis, experiments using other *Haliotis* species should be conducted.

## H. Summary

This study is the first to provide evidence that *Haliotis kamtschatkana* exhibits an alarm response to the scent of damaged conspecific tissue and to mucus actively released by handled conspecifics under laboratory and field conditions. It is also one of a very few studies that provides concrete evidence of an actively released alarm substance. The alarm response may serve to reduce predator efficiency, but this was

not confirmed experimentally. This study has also shown that the source of the alarm stimulus is associated with the mucus producing hypobranchial gland. The alarm substance is probably multifunctional and serves as more than just as a conspecific warning device. *H. kamtschatkana* has probably evolved the ability to detect a metabolite associated with the hypobranchial gland that may serve other functions in the living animal. There is evidence to suggest that heterospecific responses to damaged tissue exist. These responses may be due either to ecological or taxonomic relationships, or a combination of both.

## LITERATURE CITED

Alexander, J. E. and A. P. Covich. 1991. Predator avoidance in the freshwater snail *Physella virgata* to the crayfish *Procambarus simulans*. *Ocealogia*. (in press).

Appleton, R. D. and A. R. Palmer. 1988. Water-borne stimuli released by predatory crabs and damaged prey induce more predator-resistant shells in a marine gastropod. *Proc. Natl. Acad. Sci. USA*. 85: 4387-4391.

Atema, J. and G. D. Burd. 1975. A field study of chemotactic responses of the marine mud snail, *Nassarius obsoletus*. *J. Chem. Ecol.* 1(2):243-251.

Atema, J. and D. Stenzler. 1977. Alarm substance of the marine mud snail *Nassarius obsoletus*: Characterization and possible evolution. *J. Chem. Ecol.* 3:173-187.

Branch, G. M. 1981. The biology of limpets: Physical factors, energy flow and ecological interactions. *Oceanogr. Mar. Biol. Annu. Rev.* 19:235-379.

Bullock, T. H. 1953. Predator recognition and escape responses of some intertidal gastropods in the presence of starfish. *Behavior*. 5(2):130-140.

Burghardt, G. M. 1970. Defining 'communication' *in*: *Advances in chemoreception. I. Communication by chemical signals*. J. W. Johnson, D. G. Moulton, and A. Turk (eds.), Appleton-Century-Crofts, New York.

Cox, K. W. 1962. California abalones, Family Haliotidae. *Calif. Dep. Fish Game Fish. Bull.* 118:133.

Crowl, T. A. and A. P. Covich. 1990. Predator-induced life-history shifts in a freshwater snail. Science. 247:949-951.

Davies, M.S., H. D. Jones and S. J. Hawkins. 1990. Seasonal variation in the composition of pedal mucus from *Patella vulgata* L. *J. Exp. Biol. Ecol.* 144:101-112.

Emmet, B. and G. S. Jamieson. 1988. An experimental transplant of prerecruit abalone in Barkley Sound, British Columbia. *Fish. Bull.* 87:95-104.

Feder, H. M. 1972. Escape responses in marine invertebrates. *Scient. Amer.* 227(1):92-100.

Feder, H. M. and A. M. Christensen. 1966. Aspects of asteroid biology. pp. 87-127. *In*: *Physiology of Echinodermata.* R. A. Boolootian (ed.), Intersci. Publ. (Wiley), New York.

Fretter, V. and A. Graham. 1962. *British prosobranch molluscs*. Ray Soc. Publ., London. 755pp.

Gore, R. P. 1966. Observations on the escape response in *Nassarius vibex* (Say), (Mollusca: Gastropoda). *Bull. Mar. Sci.* 16(3):423-434.

Grenon, J. F. and G. Walker. 1980. Biochemical and rheological properties of the pedal mucus of the limpet, *Patella vulgata* L. *Comp. Biochem. Physiol.* 66B:451-458.

Hadlock, R. P. 1980. Alarm response of the intertidal snail *Littorina littorea* (L.) to predation by the crab *Carcinus maenas* (L.). *Biol. Bull.* 159:266-279.

Hartwick, B., L. Tullock, and S. Macdonald. 1981. Feeding and growth of *Octopus dofleini* (Wulkner). *Veliger*. 24:129-138.

Howe, N. R. and Y. M. Sheikh. 1975. Anthopleurine; a sea anemone alarm pheromone. *Science*. 189:386-388.

Hughes, R. N. 1986. *A functional biology of marine gastropods*. Johns Hopkins Univ. Press, Baltimore, Maryland. 245 pp.

Hunt, S. 1970. *Polysaccharide-protein complexes in invertebrates*. Academic Press, London. 329 pp.

James, M. A. and T. G. Nolen. 1978. Escape responses of *Haliotis kamtschatkana* (gastropoda) to three predatory asteroids. *Amer. Zool.* 18(3):675.

Kohn, A. J. 1961. Chemoreception in gastropod molluscs. *Amer. Zool.* 1(2):291-308.

Lawrence, J. M. 1991. A chemical alarm response in *Pycnopodia helianthoides* (Echinodermata: Asteroidea). *Mar. Behav. Physiol.* 19:39-44.

Mackie, A. M. and P. T. Grant. 1974. Interspecies and Intraspecies chemoreception by marine invertebrates. pp. 105-141. *In*: *Chemoreception in marine organisms*. Mackie, A. M. and P. T. Grant (eds.), Academic Press, London.

Mann, K. H., J. L. C. Wright, B. E. Welsford, and E. Hatfield. 1984. Responses of the sea urchin *Strongylocentrotus droebachiensis* (O. F. Muller) to water-borne stimuli from potential predators and and potential food algae. *J. Exp. mar. Biol. Ecol.* 79:233-244.

Martel, A. 1990. Recruitment, post-metamorphic drifting and reproductive output in the herbivorous gastropod *Lacuna* spp. within kelp canopies and intertidal seaweed communities. Ph. D. Theisis. University of Alberta. 329pp.

Minchin, D. H. 1975. The righting response in Haliotids. *Veliger*. 17(3):249-51.

Montgomery, D. H. 1967. Responses of two Haliotid gastropods (Mollusca), *Haliotis assimilis* and *Haliotis rufescens*, to the forcipulate asteroids (Echinodermata), *Pycnopodia helianthoides* and *Pisaster ochraceus*. *Veliger*. 9(4):359-368.

Palmer, A. R. 1990. Effect of crab effluent and scent of damaged conspecifics on feeding, growth, and shell morphology of the Atlantic dogwhelk *Nucella lapillus* (L.) *Hydrobiologia*. 193:155-182.

Parker, D. A. and M. J. Shulman. 1986. Avoiding predation: Alarm responses of Caribbean sea urchins to simulated predation on conspecific and heterospecific sea urchins. *Mar. Biol.* 93:201-208.

Parsons, D. W. and D. L. MacMillan. 1979. The escape response of abalone (Mollusca, Prosobranchia, Haliotidae) to predatory gastropods. *Mar. Behav. Physiol.* 6:65-82.

Paul, A. J. and J. M. Paul. 1981. Temperature and growth of maturing *Haliotis kamtschatkana* Jonas. *Veliger*. 23:303-309.

Pfeiffer, W. 1962. The fright reaction of fish. *Biol. Rev.* 37:495-511.

Pfeiffer, W. 1963. Alarm substances. *Experientia*. 19:113-123.

Pfeiffer, W. 1974. Pheromones in fish and amphibia. *In*: *Pheromones: Frontiers of biology*, Martin C. Birch (ed.), Vol. 32. North-Holland Pub. Co., Amsterdam. 495 pp.

Phillips, D. W. 1977. Avoidance and escape responses of the gastropod mollusc *Olivella biplicata* (Sowerby) to predatory asteroids. *J. Exp. Mar. Biol. Ecol.* 28:77-86.

Sleeper, H. L., V. J. Paul, and W. Fenical. 1980. Alarm pheromones from the marine opisthobranch *Navanax inermis*. *J. Chem. Ecol.* 6:57-70.

Sloan, N. A. and P. A. Breen. 1988. Northern abalone, *Haliotis kamtschatkana*, in British Columbia: Fisheries and synopsis of life history information. *Can. Spec. Publ. Fish. Aquat. Sci.* 103:46 p.

Smith, R. J. F. 1977. Chemical communication as adaptation: Alarm substance of fish. pp. 303-320. *In*: *Chemical signals in vertebrates*. D. Muller Schwarze and M. M. Mozell, (eds.). Plenum, New York.

Smith, R. J. F. 1982. The adaptive significance of the alarm substance-fright reaction system. pp. 327-342. *In*: *Fishes*. T. J. Hara (ed.) Elsevier Scient. Pub. Co., Amsterdam.

Smith, R. J. F. 1986. Evolution of alarm signals: Role of benefits of retaining group members of territorial neighbors. *Am. Nat.* 128(4):604-610.

Snyder, N. F. R. 1967. An alarm response of aquatic gastropods to intraspecific extract. *Mem. Cornell Univ. Exp. Stn.* 403:1-126.

Snyder, N. F. R. and H. Snyder. 1970. Alarm response of *Diadema antillarum*. *Science*. 168:276-278.

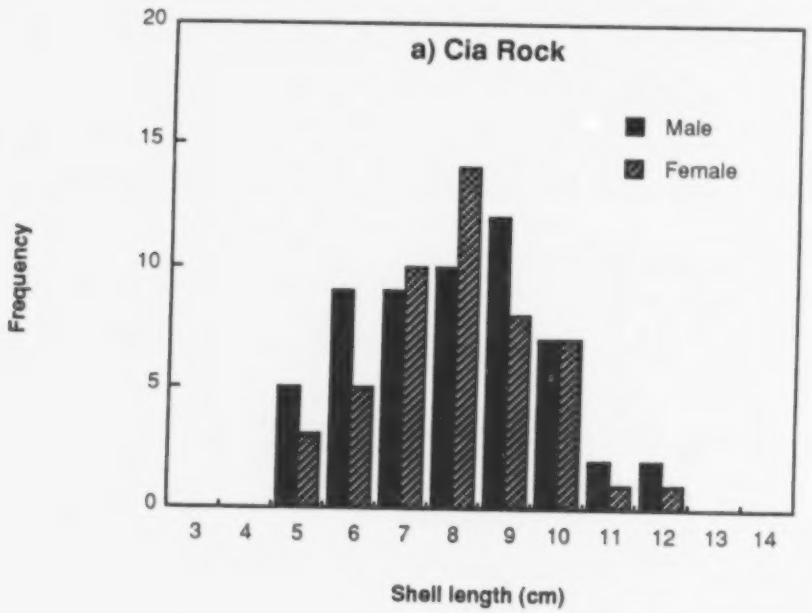
Stenzler, D. and J. Atema. 1977. Alarm response of the marine mud snail, *Nassarius obsoletus*: Specificity and behavioural priority. *J. Chem. Ecol.* 3(2):159-171.

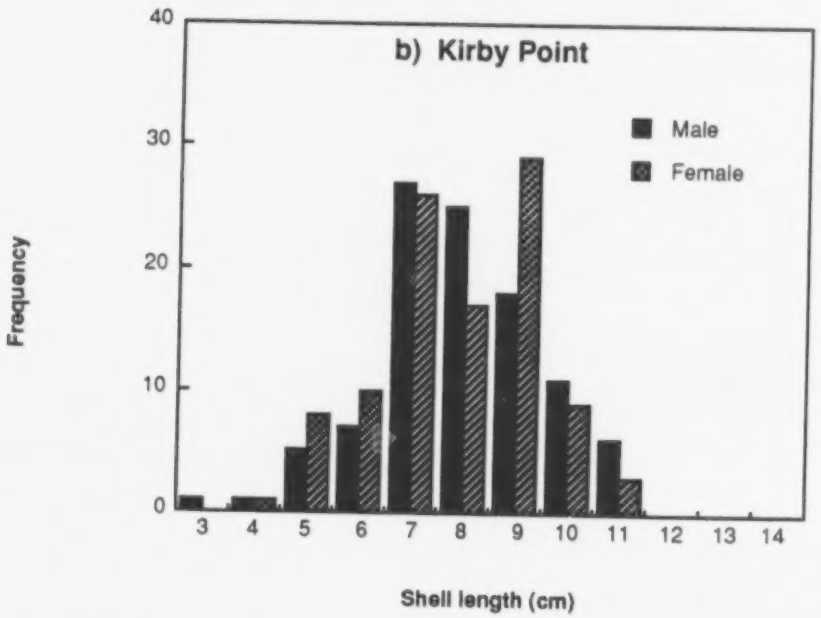
Underwood, A. J. 1979. The ecology of intertidal gastropods. *Adv. Mar. Biol.* 16:111-210.

Voltzow, J. 1986. Changes in pedal intramuscular pressure corresponding to behaviour and locomotion in the marine gastropods *Busycon contrarium* and *Haliotis kamtschatkana*. *Can. J. Zool.* 64:2288-2293.

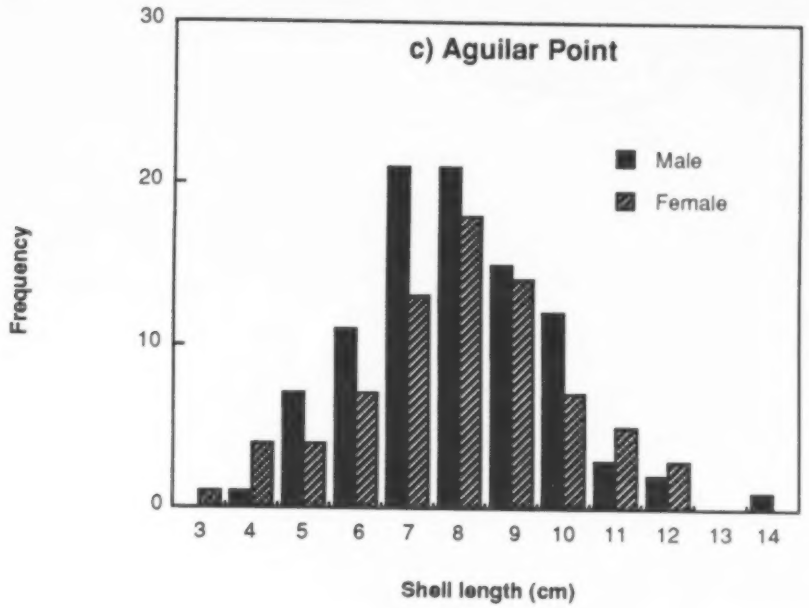
Zar, J. H. 1984. *Biosatatistical Analysis*. 2nd. ed. Prentice-Hall, N. J. 718 pp.

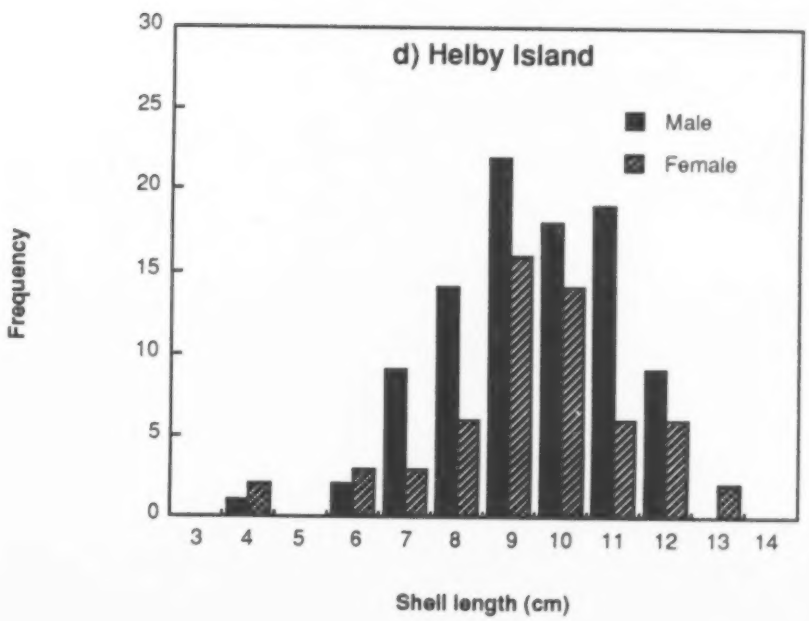
Appendix I. Shell length frequencies of *Haliotis kamtschatkana* collected for laboratory study at five different sites in Barkley Sound.



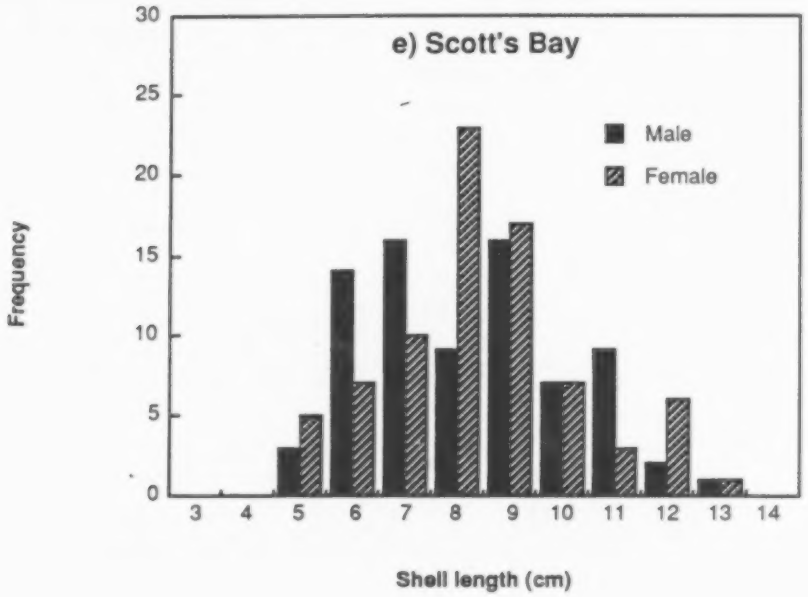



Appendix I continued

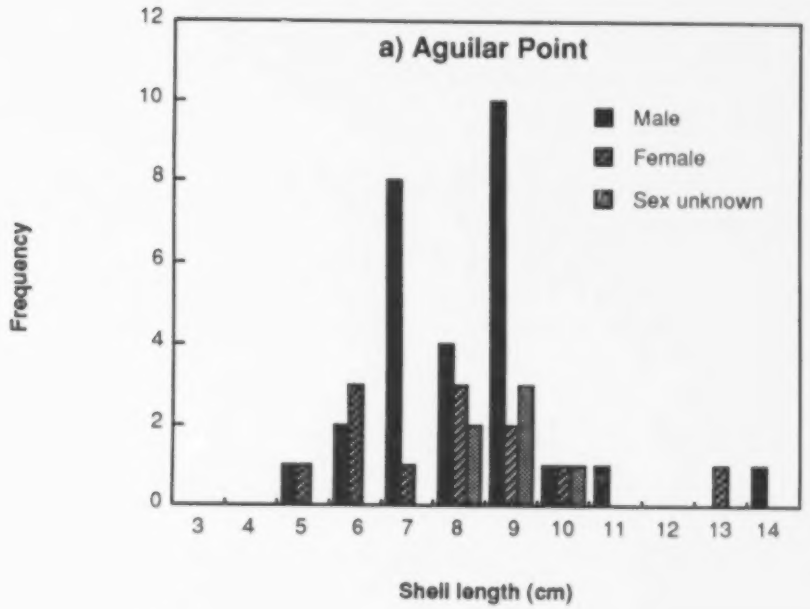


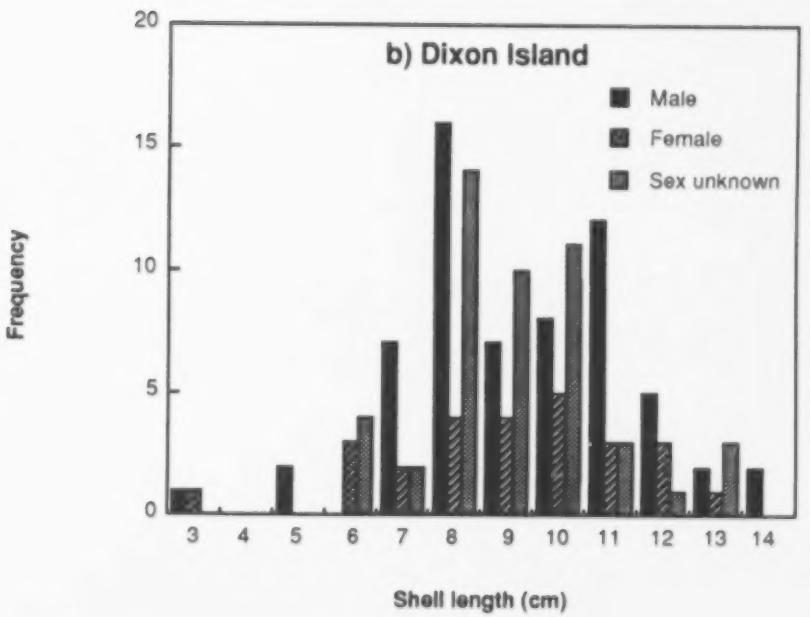



Appendix 1 continued

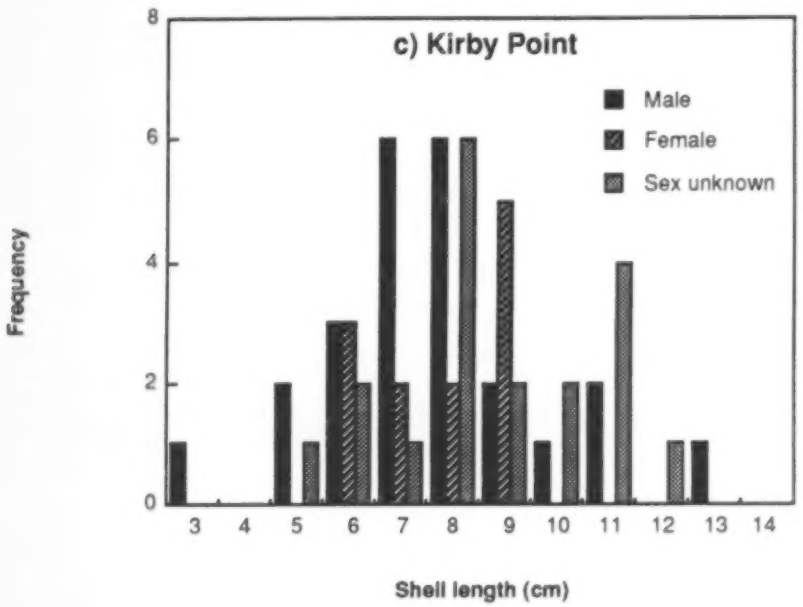


Appendix II. Shell length frequencies of *Haliotis kamtschatkana* used for field tests at three different sites in Barkley Sound.

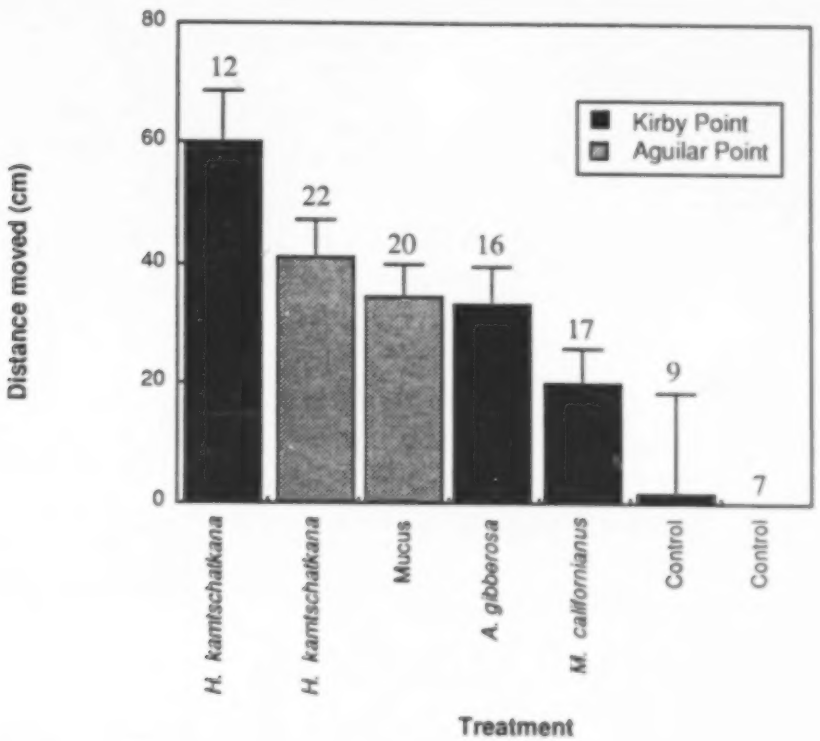


Appendix II continued.



Appendix III. Mean (± SE) distance (cm) moved over two minutes following exposure to damaged conspecific and heterospecific tissues at Kirby Point and Aguilar Point. The numbers above each bar indicate the number of abalones tested at each site.



Appendix IV. Summary of pre-experimental trials with damaged conspecific tissue.

| Date collected (day/mo./yr.) | Collection site | No. collected | Pre-test? | No. tested | No. responded | Experiment following pre-test | Pre-test Data reported in |
|---|---|---|---|---|---|---|---|
| 30/10/89 | Kirby Pt. | 75 | No+ | 40 | — | Concentration threshold (preliminary data-not used) | NA |
| 3/11/89 | Aguilar Pt. | 60 | No+ | 60 | — | Concentration threshold (preliminary data-not used) | NA |
| 10/11/89 | Aguilar Pt. | 30 | No+ | 30 | — | Concentration threshold (preliminary data-not used) | NA |
| 10/1/89 | Cia Rk. | 60 | No++ | 60 | — | Individual diffs./habituation/ laboratory effects (Figs. 4 and 5) | NA |
| 12/2/89 | Kirby Pt. | 51 | Yes | 40 | 29 | Species specificity (Fig. 10) | Fig. 2, Table 2 |
| 22/2/90 | Cia Rk. | 57 | Yes | 40 | 25 | Source of stimulus (preliminary data-not used) | Fig. 2, Table 2 |
| 10/4/90 | Helby Is. | 92 | Yes | 80 | 23 | Source of stimulus (Fig. 6, Table 5) | Fig. 2, Table 2 |

Appendix IV continued.

| Date collected (day/mo./yr.) | Collection site | No. collected | Pre-test? | No. tested | No. responded | Experiment following pre-test | Pre-test Data reported in |
|---|---|---|---|---|---|---|---|
| 18/5/90 | Helby Is. | 91 | Yes | 80 | 36 | Source of stimulus (Fig. 7, Table 5) | Fig. 2, Table 2 |
| 16/6/90 | Aguilar Pt. | 102 | Yes | 80 | 43 | Source of stimulus (Fig. 8, Table 5) | Fig. 2, Table 2 |
| 20/7/90 | Scott's Bay | 92 | No++ | 80 | — | Individual diffs./habituation/ laboratory effects (Data not used) | NA |
| 15/10/90 | Scott's Bay | 88 | Yes | 80 | 24 | Chemical characterization of stimulus (Data not used) | Fig. 2, Table 2 |
| 6/11/90 | Kirby Pt. | 90 | Yes | 80 | 42 | Chemical characterization of stimulus (Data not used) | Fig. 2, Table 2 |

+ Preliminary experiments conducted prior to the implementation of pre-trials.

++ Pre-trials were not run prior to these tests.